Statistical optimization of alginate-based oral dosage form of 5-aminosalicylic acid aimed to colonic delivery: in vitro and in vivo evaluation

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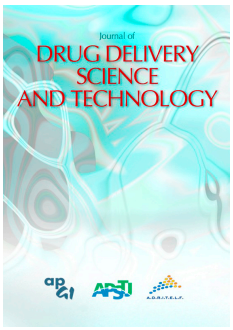

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Ehsan Kaffash, Farinaz Saremnejad, Mohammadreza Abbaspour, Seyed Ahmad Mohajeri, Hadi Afrasiabi Garekani, Amir Hossein Jafarian, Hossein Shahdadi Sardo, Abbas Akhgari, Ali Nokhodchi

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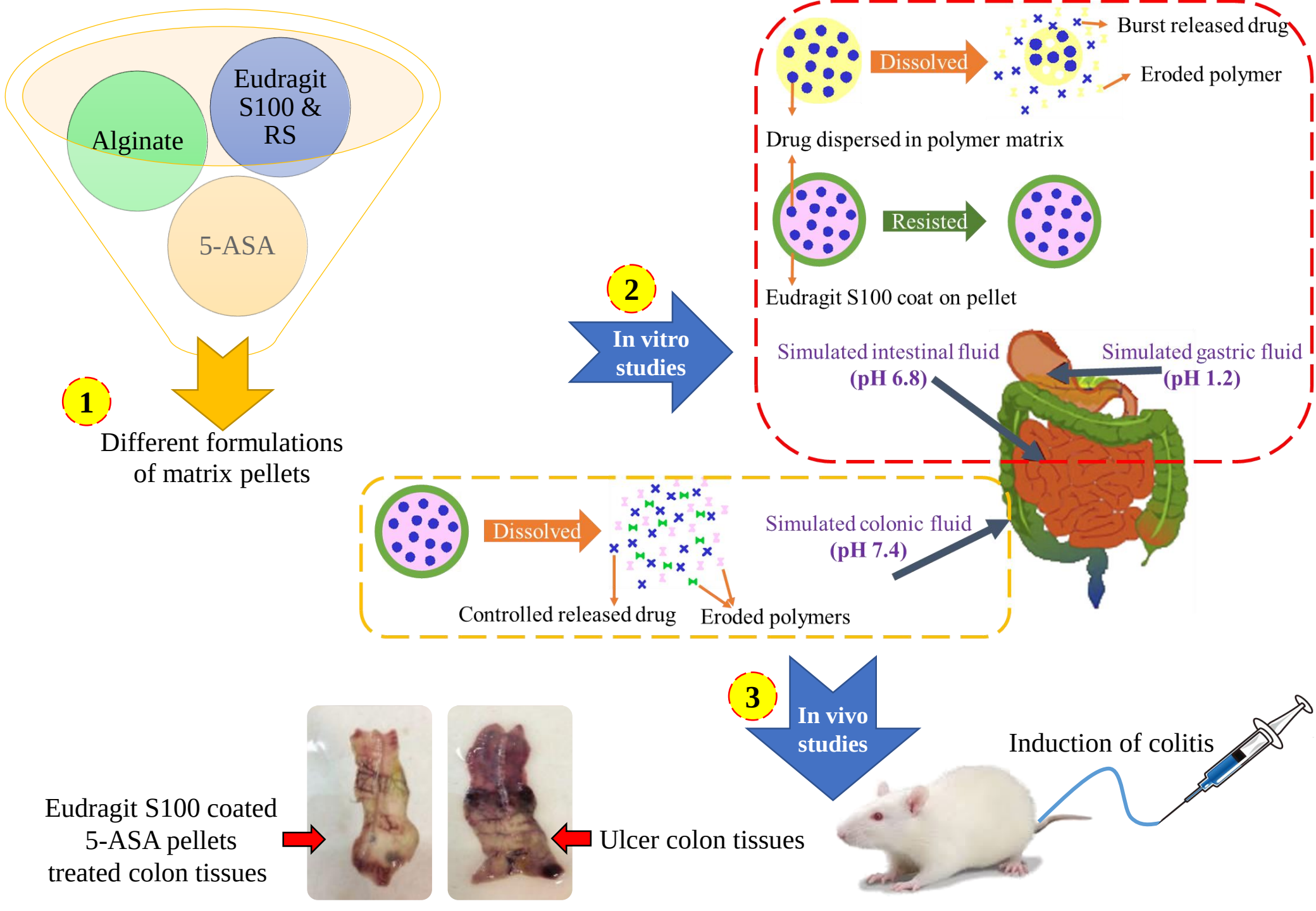
Alginate
Eudragit S100 & RS
5-ASA
1
Different formulations of matrix pellets
2
In vitro studies
Dissolved
Burst released drug
Eroded polymer
Drug dispersed in polymer matrix
Resisted
Eudragit S100 coat on pellet
Simulated intestinal fluid (pH 6.8)
Simulated gastric fluid (pH 1.2)
Dissolved
Simulated colonic fluid (pH 7.4)
Controlled released drug
Eroded polymers
3
In vivo studies
Induction of colitis
Eudragit S100 coated 5-ASA pellets treated colon tissues
Ulcer colon tissues

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Ehsan Kaffash [a,b], Farinaz Saremnejad [c], Mohammadreza Abbaspour[a, d], Seyed Ahmad Mohajeri[d, e], Hadi Afrasiabi Garekani[a, d], Amir Hossein Jafarian [f], Hossein Shahdadi Sardo[a, d], Abbas Akhgari[a, d*], Ali Nokhodchi[g,*]

[a]Department of Pharmaceutics, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran; [b]Student Research Committee, Mashhad University of Medical Sciences, Mashhad, Iran; [c]Department of Food Science and Technology, Ferdowsi University of Mashhad, Mashhad, Iran; [d]Targeted Drug Delivery Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran; [e] Department of Pharmacodynamics and Toxicology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran; [f]Cancer Molecular Pathology Research Center, Faculty of Medicine, Ghaem Hospital, Mashhad University of Medical Sciences, Mashhad, Iran; [g]Pharmaceutics Research Laboratory, School of Life Sciences, University of Sussex, Brighton, UK

* Corresponding authors:

Abbas Akhgar, akhgaria@mums.ac.ir (Department of Pharmaceutics, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran ; +98 51 31801327)

Ali Nokhodchi, a.nokhodchi@sussex.ac.uk (Pharmaceutics Research Laboratory, School of Life Sciences, University of Sussex, Brighton, UK +44 1273872811)

Abstract

Although different colon-targeting methods such as pH and time-dependent as well as bacterially degradable systems have been developed, due to variations in physiological conditions of patients, one system alone cannot produce a reliable drug delivery system. Therefore, in this study, an attempt was made to formulate 5-ASA pellets based on a combination of pH and time-dependent systems. A 3^2 full-factorial design was used to evaluate the effect of sodium alginate (SA) concentration and the polymer ratio (Eudragit® RS: Eudragit® S100) on morphology, mechanical features and release behaviour of 5-ASA pellet formulations. Fourier transform infrared spectroscopy, and differential scanning calorimetry analysis ruled out any interactions between the formulations components. The optimized formulation released 35, 55 and 89% of the drug within 15 h, at pHs 1.2, 6.8 and 7.4, respectively. *In vivo* studies demonstrated that the administration of sodium alginate-based matrix pellets containing 5-ASA coated with ES100 was effective in alleviating the damaged tissues and also decreased the inflammatory score and the rate of weight loss in colitis rats. The results indicated the remarkable anti-inflammatory efficacy of the designed colonic delivery system and the proposed formulations can be further developed in the pharmaceutical industry for patient use.

Keywords: 5-aminosalicylic acid, Alginate, Eudragit® RS, Eudragit® S100, Pellet, *In vivo*

1. Introduction

5-aminosalicylic acid (5-ASA) is an anti-inflammatory drug, which is commonly used in the treatment of inflammatory bowel diseases (IBD) such as ulcerative colitis (UC) and Crohn's disease (CD) [1]. But most of orally administered 5-ASA molecules are capable of being absorbed or degraded in the stomach and small intestine before they reach the colon [2]. In recent years, it has become possible to deliver drugs to the colon in a specific manner. Colon-specific delivery systems could prevent the release of the drug in the upper part of the gastrointestinal tract (GIT), and they use a triggering mechanism that causes sudden drug release upon reaching the colon [3]. Many studies have been focused on the design of colon-targeted drug delivery system (CDDS), which drug release mechanisms are based on a physiological characteristic of GIT such as pH, time and bacterial or combinations of them [4–7].

pH-dependent systems utilize the pH changes along the GIT to control drug release. Eudragit® S100 (ES100) is an anionic pH-dependent copolymer based on methacrylic acid and methyl methacrylate (1:2 ratio). ES100 is insoluble in acids and pure water, whereas soluble in aqueous solution at pH 7.0 or higher [8]. It is reported that ES100 as a pH-sensitive matrix former influenced the pellet size and roundness, and also failed to delay the drug release [9]. However, 5-ASA pellets coated with ES100 were resistant to gastric and upper intestinal pH while quickly released the drug at pH>6.8 [10].

Furthermore, natural polymers with acidic or basic groups have been employed as carriers for colon-specific drug delivery due to their non-toxicity, biocompatibility and easy availability [11]. Alginate is a natural polymer that has been widely used as drug carriers for oral administration [12]. Also, the potential therapeutic effect of sodium alginate (SA) in colitis rats in reducing inflammation was reported [13,14]. The presence of carboxylic groups in alginate structure makes it very sensitive to the pH changes in the surrounding environment

of this polymer. Theoretically, alginate shrinks at low pH of the gastric environment and the drug cannot be released [15]. Therefore, pH-responsiveness of this polymer has been exploited for the development of oral colon-specific drug delivery devices [16]. Time-dependent systems are designed to achieve colon-specific delivery by utilizing the transit time for the formulation to pass through the upper GIT. Eudragit® RS (ERS) is an acrylic polymer that swells and releases the incorporated drug via diffusion and erosion. Its combination with other polymers in matrix pellets could relatively control drug release in the upper GIT [17].

Various dosage forms have been developed to provide controlled release and colon-targeted delivery of 5-ASA. Multi-particulate dosage forms (e.g., pellets, granules, or microparticles) offer several advantages such as more homogenous drug distribution and better drug absorption compared to single-unit dosage forms (e.g., tablets or capsules) [18]. Pellets are spheres with a diameter of 500-1500 μm that have several technological and therapeutical advantages including enteric protection of the particles or controlling the drug release, and wide dispersion in the GIT. Among the techniques employed for preparing pellets, extrusion-spheronization has gained particular attention due to the highly uniform particle size distribution of the resultant pellets [19]. These pellets can be produced as matrix pellets with the appropriate polymer and are coated in the fluid-bed unit to provide modified release dosage forms [20].

Although the *in vitro* release conditions can potentially be crucial for the observed drug release the *in vivo* studies of this type of dosage forms also seems necessary [21]. For instance, *in vivo* studies showed that the administration of chitosan pellets containing 5-ASA coated by pH-sensitive Eudragit® FS which controlled the delivery of the drug to the colon decreased the colon/body weight ratio in colitis rats [22]. In another study, the rat model of ulcerative colitis showed the optimal therapeutic effect when 5-ASA pellets containing

Surelease® and Eudragit® S100 as a drug carrier [23]. Similar results observed for coated pellets of 5-ASA in different studies [24,25].

The aim of this study was to design pH and time-dependent 5-ASA matrix pellets containing SA, ERS, and ES100 with a capability to release the dug at the desired site of the action when is tested on colitis rats. To this end, a 3-level full factorial design was employed to explore the process variables involved in the optimization of the pellets formulations. The effect of the explored factors was also investigated on the pellets characterization, mechanical parameters as well as the dissolution behaviour of the drug from the optimized pellets.

2. Materials and methods

2.1. Materials

5-ASA was purchased from Arya pharmaceutical co. (Tehran, Iran), microcrystalline cellulose (Avicel® PH101) were provided by Darupakhsh (Tehran, Iran), calcium carbonate and potassium phosphate monobasic were supplied from Merck (Germany), sodium alginate (number-average molecular weight 12,000–40,000 and viscosity 5.0-40.0 cps) was purchased from Sigma-Aldrich (USA), Eudragit® RS PO and Eudragit® S100 were provided from Evonik® (Germany). All excipients used to prepare pellets and coating were of standard pharmaceutical grade, and all chemical reagents used were of analytical grade.

2.2.Preparation of pellets

The solid components of each formulation (Table 1) were mixed for 10 min, and then sufficient amount of water as a granulating liquid (this was not measured) was added slowly to the mixture to make a wet mass (past-like) with a suitable consistency. The wet mass was passed through an axial screw extruder (type HC 732, Dorsa, Iran) with a 1 mm screen at 100 rpm. The extrudates were rounded by a spheronizer (type HC 732, Dorsa, Iran) with a cross-

hatched plate at 1000 rpm for 5 min. The obtained pellets were dried and cured at 70 °C in an oven (Parseh ON-12, Iran) for 7 h [17].

The pellets were coated in a fluidized-bed coating apparatus (Wurster insert, Werner Glatt, Germany). ES100 solution (10% w/v) was prepared in isopropyl alcohol:water (9:1) mixture. Then, 10% (w/w) dibutyl phthalate related to the dry polymer as a plasticizer was added directly to the polymer dispersions. This was followed by the addition of 5% w/w talc (related to dry polymer) as a glidant. The inlet air temperature was set at 40 °C, and the outlet temperature was in the range of 25–35 °C. The atomizing pressure was 2.0 bar, and the spray rate was 10 g/min. 200 g of 5-ASA pellets were coated in fluidized-bed coating apparatus to achieve a 10% (w/w) weight gain. The pellets were fluidized for an extra 5 min, and samples were kept in an oven for 2 h at 40 °C [26].

2.3.Morphology of pellets

Pellets were sieved with standard sieves mesh size 16 and 35 and shaken for 5 min. The pellets that remained on the sieve with mesh 35 (size range 500-1180 μm) were considered as appropriate pellets, and the weight of pellets in this range was reported as the yield of pellets.

Microscopic image analysis was used to determine the shape and size of the pellets. About 20 pellets from each batch were attached on a black matt background, and a top light source was used to remove the influence of shadow on the image processing. Image analyzer consisted of a stereomicroscope (Olympus, DP25, Okura, Japan) at magnification (8.5×) and a digital camera (Sony, Japan). Digitized images were analyzed by the image analyzing software (ImageJ 1.50f for Windows). The area (A), perimeter (p_m) and the longest and shortest Feret's diameters (d_{max} and d_{min}) of pellets were measured, and two shape factors were calculated as followed [27]:

$$\text{Aspect ratio} = d_{max} / d_{min} \quad (1)$$

Sphericity = $4\pi A / p^2_m$ (2)

Also, surface characteristics of pellets were examined by scanning electron microscope (SEM) (LEO1450 vp, Zeiss Company, Germany).

2.4.Mechanical tests

The crushing strength (CS; force needed to break the pellets) of 20 pellets (500-1180μm size fraction) was measured using a testing machine equipped with a 1kN load cell (Hounsfield-H50KS, United Kingdom). The speed of the upper mobile plate was set at 1 mm/min [28].

2.5.Fourier transform infrared spectroscopy

FT-IR spectroscopy was accomplished in KBr discs over a range of 4000–500 cm^{-1} using IR spectroscope equipment (vortex 70, Bruker, Germany).

2.6.Differential scanning calorimetry

Differential scanning calorimetry was performed by DSC (Metler Teldo DSC822, Switzerland) to evaluate thermal behaviour of the pellets and also to investigate any interaction between the excipients and the drug used in the formulation of the pellets. The samples were heated from 50 to 250 °C at a heating rate of 10 °C/min.

2.7.Determination of drug content in pellets

To determine the drug content in pellets, accurately a certaine amount of pellets (250 mg which is equivalent to the 50 mg drug) was added to 250 mL of phosphate buffer pH 7.2 in a volumetric flask. The volumetric flask was shaken in water bath at 37 °C for 10 hr. Dilutions were made suitably and measured for the drug content by UV spectrophotometry at 330 nm. Assays were carried out in triplicate and the mean value was reported.

2.8.In vitro dissolution studies

Dissolution studies of uncoated and coated pellets were done with a basket method (USP apparatus I) in the 900 mL medium at 37 °C at a rotation speed of 100 rpm (Pharmatest, Germany). An accurate amount of pellets equivalent to 50 mg of 5-ASA were weighed and transferred to the basket of dissolution medium. The dissolution tests were performed for 2 h in pH 1.2 (HCl 0.1N) and 10 h in the phosphate buffer media (pHs 6.8 and 7.4). The samples were taken from the vessel by a peristaltic pump (Alitea. Sweden) and passed through a multi-cell system on the UV spectrophotometer (Shimadzu, Japan) and assayed at 302 nm (for acidic media) and 330 nm (for buffer media) and returned to the vessel (n=3).

From drug release data, mean dissolution time (MDT) was computed via the following equations [29]:

$$MDT = \frac{\sum \bar{t}_i \,.\, \Delta M_i}{\sum \Delta M_i} \quad (3)$$

$$\bar{t}_i = \frac{t_i + t_{i+1}}{2} \quad (4)$$

$$\Delta M_i = M_{i+1} - M_i \quad (5)$$

Where $\bar{t}_i$ is the midpoint of the time during which the fraction ΔM_i of the drug released from the dosage form. A high MDT value means that the drug delivery system has a slow *in vitro* drug release.

The continuous dissolution test was performed for the most promising formulation (F11 coated with 10% ES100) based on accepted GIT times; 2 h for pH 1.2 (simulating gastric fluid), 3 h for pH 6.8 (simulating Duodenum) and 10 h for pH 7.4 (stimulating lower small intestine and colon), respectively. The 5-ASA cumulative release percentage was calculated over the sampling times.

2.9.Experimental design

A 3^2 full factorial design was used for the optimization procedure. The independent variables were SA concentration (X_1) and ERS/ES100 ratio (X_2), each at three different levels as shown in Table 2. The dependent variables were aspect ratio (Y_1), sphericity (Y_2), CS (Y_3), MDT_a in pH 1.2 (Y_4) and MDT_b in pH 6.8 (Y_5).

The effect of independent variables on the responses was modeled with the help of Design-Expert® software version 11.0.3.0 (Stat-Ease, Inc., USA) using following polynomial equation:

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_{11} X_1^2 + b_{22} X_2^2 + b_{12} X_1 X_2 \quad (6)$$

Where Y is the measured response, X_i is the level of the *i*th factor; b_0 represents the intercept, and b_i, b_{ij} ... represent coefficients computed from the responses of the formulations in the design.

ANOVA was applied to further reducing the generated polynomial equations on the basis of significant terms. The 3^2 full factorial design was validated for selected formulations. The predicted values for CS, MDT_a, and MDT_b were determined on the basis of respective polynomial equations whereas the experimental (observed) values were determined by evaluating formulations for the selected dependent variables. The predicted and observed values of the responses were compared for statistical significance using a paired t-test.

2.10.*In vivo* studies

Male Wistar rats (280±40 g), aged 8–12 weeks, were housed in 12 h light/dark cycles at 22±3 °C and allowed free access to water and standard laboratory food duration of the studies (the standard laboratory food is oval pellet, 10 mm × 16 mm × 25 mm length, so that the animals' requirements such as energy, protein, calcium, phosphorus, methionine, and lysine could be

attained. This oval pellet contains ground corn, dehulled soybean meal, fish meal, wheat middlings, cane molasses, dehydrated alfalfa meal, soybean oil, ground oats, dried beet pulp, wheat germ, brewers dried yeast, dicalcium phosphate, calcium carbonate, salt, DL-methionine, choline chloride, cholecalciferol, menadione dimethylpyrimidinol bisulfite (source of vitamin K), pyridoxine hydrochloride, vitamin A acetate, biotin, thiamine mononitrate, vitamin B12 supplement, dl-alpha tocopheryl acetate (form of vitamin E), nicotinic acid, calcium pantothenate, riboflavin supplement, manganous oxide, zinc oxide, ferrous carbonate, copper sulfate, zinc sulfate, calcium iodate, cobalt carbonate). Rats fasted for 24 h with free access to water before the induction of colitis. The experimental method involving animals was conducted in accordance with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). It was also approved by the Institutional Animal Care and Use Committee, Mashhad University of Medical Sciences, Mashhad, Iran, with the ethical number IR.MUMS.SP.1395.63.

2.10.1. Induction of colitis

Inflammatory lesions were induced in a method described by Millar *et al*. [30]. Each rat was anaesthetized by ketamine/Xylazine (1:3, v/v), received 2 mL acetic acid 3% (prepared in normal saline 0.9%), and through anus using a polyethylene tube (2 mm in diameter) which inserted 8cm into the colon and the fluid was withdrawn after the 30s.

2.10.2. Treatment and experimental design

After 5 days of colitis induction, rats were randomly divided into four groups (5 animals in each group): normal control group (colitis was not induced), negative control group (no drug was administered after colitis induction), vehicle-treated group (1% Na-CMC), positive control group (received 5-ASA pellets with vehicle) and ES100 coated 5-ASA pellets

(received with vehicle) treated group (Figure 1). A dose of 120 mg/kg/day of 5-ASA calculated from the dose from humans (70kg) [31] was administered by oral gavage once a day for five consecutive days. Food and water were allowed throughout the treatment. After five days the rats were sacrificed, and the colon segments were removed. The damage of the colon sample was evaluated by macroscopic scoring and histological examination. The weight-loss of each group was tracked every day after colitis induction and also their weight-gain was recorded after the onset of the treatment. Rats that had more than 80% weight loss (weight less than 195 g) were omitted from the study, also rats with bloody diarrhoea, prolonged prostration and fever were excluded.

2.10.3. Assessment of colitis

Macroscopic scoring of the colon (8 cm), extending proximally for 2cm above the anus was removed. The colonic tissues were preserved in formalin (10% v/v) solution to prevent autolysis and were split longitudinally. The macroscopic assessment of the colonic damage was scored according to Table 6 criteria which were reported elsewhere [32]. The histopathological study was performed on sections of colon tissues. Cross-sections of colon segments were fixed in 10% buffered formalin solution (pH 7.4). The sections were stained with haematoxylin and eosin (H&E) followed by microscopic observations [33]. Histological evaluation of colonic damage was assessed by a light microscope equipped with a digital camera.

2.11.Statistical analysis

Data were expressed as means ± SD. For statistical evaluations of histological results, non-parametrical ANOVA (Kruskal-Wallis test) was used, and one-way ANOVA followed by

Tukey test was applied for comparing body weights. A value of p-value<0.05 was considered significant.

3. Results and discussion

3.1. Characteristics of pellets

The content of 5-ASA in pellet, as estimated by UV spectrophotometry, was 18.7 ± 2 % w/w which indicated the coherent distribution of the drug by extrusion-spheronization process. The results of the yield of different formulations of pellets showed that approximately 88% of the uncoated pellets were within the desired size range of 500–1180µm (Table 3). Also, image analysis results revealed that the aspect ratio and sphericity of pellets were near to 1 (Table 3) which shows that SA concentration has not affected these parameters and the extrusion-spheronization process provides almost spherical pellets. Similarly, Charoenthai *et al*. produced spherical pellets by including SA in the formulations [34].

SEM data were used to confirm the results of image analysis (aspect ratio and sphericity) as well as the surface of uncoated and coated pellets (Figure 2). The surface of the uncoated pellets was smooth, and also, the coated pellets show that the coating layer is uniform and formed continuously. Duan *et al*. also obtained spherical and smooth-face in shape after coating alginate based microparticulates with ES100 [35]. The cross-section of coated pellets was shown in Figure 2D and it revealed the thickness of the coating layer was about 8-10 µm. After continuous dissolution test, the uniform coating layer (Figure 2C) changed to a porous surface (Figure 2F) which facilitated the drug release throughout these pores. This was probably due to the dissolution of ES100 above pH 7.0.

3.2. FTIR

The characteristic peaks of 5-ASA spectra (Figure 3a) corresponded to 2500–3000 cm^{-1} (stretching vibrations of the hydrogen bonds), 1642 cm^{-1} (C=O stretch), 1451cm^{-1} (NH blend), 1352cm^{-1} (C–N stretch). In the case of ES100 (Figure 3b) the peak at 1729 cm^{-1} was attributed to the esterified carboxyl groups. ERS showed an ester C=O stretching peak around 1738 cm^{-1} (Figure 3c). The spectrum of alginate (Figure 3d) showed important absorption bands regarding hydroxyl, ether and carboxylic functional groups. Stretching vibrations of O–H bonds of alginate appeared in the range of 3000–3500 cm^{-1}. Stretching vibrations of aliphatic C–H were observed at 2928 cm^{-1}. The observed bands in 1650 and 1450 cm^{-1} were attributed to asymmetric and symmetric stretching vibrations of carboxylate salt ion, respectively. The presence of characteristic peaks of the drug in the FT-IR spectra of the physical mixture (pellet) indicates the absence of chemical interaction between the drug and the polymers (Figure 3e). FT-IR has been used in other studies to confirm the presence or lack of interaction between drug and polymers [36,37].

3.3. DSC

The pure 5-ASA (Figure 4a) showed a sharp endothermic peak at 284.5°C that corresponded to drug melting point due to its crystalline nature [38]. In the case of ES100 and ERS (Figure 4b, 4c), glass transition temperature region and an endothermic peak were observed at 216.2°C and 70.1°C, respectively [8,39]. The DSC trace of alginate (Figure 4d) shows a broad endothermic peak around 91.0°C and a sharp exothermic peak at 250.8°C. These peaks may be caused by the loss of water and moisture content and thermal decomposition of the polysaccharide, respectively. A physical mixture of 5-ASA, ES100, ERS and alginate (pellet) as a positive control (Figure 4e) showed a broad endothermic peak around 79.2°C which is probably related to the overlapping of the glass transition peaks of Eudragits and the loss of water and moisture content. Also, the endothermic peak of 5-ASA was absent in the DSC

315 traces which is probably due to a reduction in the crystallinity of the drug. Therefore it could
316 be concluded that the polymorphic changes ocurred during the extrusion-spheronization
317 process [8,40].
318
319 **3.4. *In vitro* dissolution studies**
320 An effective colonic drug delivery system must remain intact in the upper GIT, i.e., the
321 physiological environment of the stomach and be able to release the majority of its drug
322 content in the colonic medium [41]. The small intestine is divided into two major parts: upper
323 parts with nearly pH 6.8 and residence time of 3h, the lower part and terminal ileum with pH
324 7.4 and residence time of 1h [42]. Regarding this fact, the dissolution tests were carried out
325 using change over media (pH 1.2, 6.8 and 7.4). Primary dissolution results of the uncoated
326 pellets in pH 1.2 and 6.8 indicated that more than 90% drug released from all formulations in
327 less than 20 min (Figure 5). Similarly, a burst drug release for 5-ASA matrix pellets in pH 6.8
328 was reported which could be due to the action of the time-dependent swelling of Eudragit®
329 polymers in the matrix of pellets [4]. Hence expected controlled drug release from matrix
330 pellets could not be achieved.
331 Since the performance of formulations depends on the transit time of the system in GIT, so
332 they should be formulated by applying coats which are capable of delaying the drug release
333 [43]. So, exerting ES100 as a coating layer could be an excellent candidate to control drug
334 release. Therefore morphology, mechanical features and release behaviour of formulations
335 were further analyzed using response surface methodology for selection of optimized
336 formulation to be coated with ES100.
337 Both aspect ratio (Figure 6A) and sphericity (Figure 6B) have negligible variation from the
338 desired amount (sphericity 1 is an indication of a complete sphere), indicating that near
339 spherical pellets were obtained. Overall the results showed that SA concentration and

polymer ratio did not affect the aspect ratio and sphericity of pellets. These results were in agreement with the findings of Abbaspour *et al.* which reported that the binder concentration and type of Eudragit® did not affect the shape and sphericity of pellets [17]. Thus, among the different response variables CS, $MDT_{a,}$ and MDT_b were selected as the most representative to optimize the formulations. Mathematical relationships between the dependent and independent variables generated using Design-Expert® software, and the following equations were generated for the observed responses:

$$Y_3 = + 3.67 + 2.63\ X_1 + 2.79\ X_2 + 2.04\ X_1\ X_2 + 2.28\ X_1^2 - 2.29\ X_2^2 \quad (7)$$

$$Y_4 = + 32.17 + 0.1194\ X_1 + 0.5984\ X_2 - 1.15\ X_1\ X_2 - 3.62\ X_1^2 - 13.88\ X_2^2 \quad (8)$$

$$Y_5 = + 7.51 - 13.55\ X_1 + 6.78\ X_2 - 11.60\ X_1\ X_2 \quad (9)$$

Analysis of variance indicated that the assumed regression models were significant and valid for each considered response shown in Table 4.

Both the amount of SA and polymer ratio have a significant effect on the CS of pellets according to Eq. (7). Results indicate that by increasing the amount of SA, the hardness increases significantly (*p*-value, 0.0126). On the other hand, pellets containing a low amount of SA showed brittle behaviour under mechanical tests. However, the pellets containing 20% SA underwent a plastic deformation without any fracture (Figure 6C).

This was probably due to the cross-linking of calcium ions with the SA, which makes matrices harder [44]. A high amount of SA resulted in a sticky formulation which produces hard pellets after thermal heating. Also in the presence of high amount of ERS, the elastic modulus and mechanical strength of pellets increased due to the melting of the ERS during the thermal heating. Similar results have also been proven in previous studies [45,46].

Eqs. (8) and (9) represent the quantitative effect of the amount of SA and polymer ratio on MDT_a and MDT_b of pellets. According to Figure 6D, an increase in MDT_a was observed for the pellets containing 20% SA. That means these formulations have a slow drug release at pH 1.2. Approximately an equal ratio of SA to calcium showed optimal release resistant. It was

363 probably due to the role of calcium in controlling the matrice swelling which is shown in
364 many studies [47–49]. It should be noted that the higher amount of SA (40%) than calcium
365 maybe resulted in extreme swelling of matrices which led to a decrease of MDTa. On the
366 other hand, in matrices with a lower percentage of SA, swelling is not adequate to control the
367 drug release.
368 As seen in Figure 6E in the absence of SA, increasing the amount of ERS in pellets leads to
369 rising MDT_b. However, the presence of SA in the formulations decreases MDT_b significantly
370 (*p*-value, 0.0278) which is suitable from the point of drug release in the colon region.
371 Once again the role of calcium in controlling the matrices swelling was highlighted. High
372 water uptake of SA at pH 6.8 cause more swelling. Matrices with an equal ratio of SA to
373 calcium showed better swelling than matrices without SA or higher amount of SA in the
374 absence of calcium. According to the Figure 6E the presence of ES100 in matrix decreased
375 MDT_b but due to its negligible resistance to release in acidic medium is not desirable to use in
376 the matrix.
377 In order to check the validity of the generated equations in the optimization procedure, F2, F5
378 and F8 formulations (with different SA concentrations) were selected to a comparative
379 analysis of the observed and predicted values. No significant ($p<0.01$) difference between
380 these two values (Table 5) indicated the validity of the generated model. Eventually, these
381 formulations coat with ES100.
382 Coated formulations which after this called F10, F11 and F12, showed resistance in the acidic
383 medium. Drug release after 2h at pH 1.2 was 39.8, 29.5 and 39.8% for F10, F11, and F12
384 formulations, respectively (Figure 7A). Results are compatible with findings of other studies
385 that used the natural polymer as a core and subsequently coated with ES100 for colon
386 targeting [50–52]. ES100 coating decreased the burst release of the drug to a great extent due
387 to the coverage of pores and increased diffusion path length [41]. The increase in pH of the

dissolution media to 6.8 increased the drug release which could be due to the action of time-dependent swelling of ERS in the matrix of pellets [4]. Also, at this pH the carboxylic groups of SA became ionized (COO^-), resulting in swelling of the matrix [53]. Coated pellets containing SA (F11 and F12) released over 80% of their active ingredient after 6h (Figure 7B). The percent of drug release was significantly higher at the pH 7.4 compared to pH 6.8 (Figure 7C). It is because ES100 dissolves at pH 7.0 and forms a porous surface at the coating layer. This increased porous surface allowed entry of a large amount of dissolution media into the pellets leading to complete release of the drug [54]. Also, the increase in SA level to 40% had an effect on the drug release profiles. The release of 5-ASA from F12 completed after 6h, while 90% of the drug was released after 10h in case of F10 and F11 formulations.

Formulation F11 was selected as the optimized formulation for studying continuous dissolution which provides a realistic *in vitro* simulation of the GIT. The pH gradient of the GIT is a most crucial physiological factor considered in the design of controlled release formulations. The coatings of these formulations were designed to resist the acidic medium of the stomach and dissolve in a more basic medium of the terminal ileum (around pH 7). However, consistent release of 5-ASA from these formulations may be hindered by fluctuations of intestinal pH levels in patients with UC, in whom colonic pH levels have been measured at lower than pH 7. Also, considerable tablet-to-tablet variability in dissolution has been observed for pH-dependent release formulation such as ASACOL® at pH<7. Therefore, in patients, the release of 5-ASA may be inconsistent and unpredictable from some oral formulations [55,56]. Results from the continuous dissolution test indicated that the designed colonic formulation (F11) could overcome these challenges. F11 released the 55.31 and 89.23% of the drug at pH 6.8 and 7.4, respectively (Figure 8) so it can be used to treat a broader range of IBD patients with fluctuations of intestinal pH levels.

3.5. *In vivo* studies

In vivo study was carried out in 5 groups. After two days of colitis induction, clinical symptoms such as prostration, diarrhoea, hypomotility, and weight-loss were observed in all animals (except normal control rats). The weight-loss was monitored daily after intracolonic administration of acetic acid (Figure 9). The treatment was carried out from day 5 to day 10. According to our results, colitis induction caused a significant weight loss compared to normal rats in all groups. The weight loss was continued from day 1 to 10 in negative control and vehicle-treated groups. The weight loss in negative control and vehicle-treated groups from day 1 to 10 was about 16% and 12%, respectively. The rate of weight loss in positive control and 5-ASA ES100 coated groups decreased from day 5 to day 7. From day 7 to day 10, a small increase in weight (2-3%) was observed in the aforementioned groups which shows the efficacy of treatment strategy in the prevention of weight loss. On the final day, compared to the day 1, the percentage of the weight loss in the negative control group, vehicle-treated group, the positive control group, and 5-ASA ES100 coated group was 16%, 12%, 5%, 8%, respectively.

Histological evaluation was carried out on the final day of treatment. Representative photographs of colonic tissue indicated the anti-inflammatory benefit of 5-ASA ES100 coated pellets and positive control groups compared to other groups (Figure 10). Images of macroscopic evaluations (Figure 10, upper panel) showed a normal colon with no macroscopic damage in the normal control group. The colon tissues in negative control and vehicle-treated groups were severely damaged, and mucosal congestion, haemorrhage, ulcers, and necrosis were observed and tangible. Administration of 5-ASA and 5-ASA ES100 coated pellets significantly alleviated the damaged tissues in the positive control group and 5-ASA coated pellets group. Compared to negative control group, photographs of H&E staining

(Figure 10, below panel) of colons revealed a decrease in the extensive necrotic destruction of epithelium, oedema, and ulceration at mucosal and sub-mucosal layers in positive control and 5-ASA ES100 coated pellets groups.

A significant difference (p-value<0.001) between positive control (treated with 5-ASA pellets) and negative control groups was observed in reducing the inflammation severity in colon segment (Figure 11). The average of microscopic scores in negative control and vehicle-treated groups were 2.8±0.37 and 2.5±0.28, respectively, whereas the scores in positive control and 5-ASA ES100 coated pellets group was 0.8±0.48 and 1.8±0.23, respectively. The treatment with ES100 coated 5-ASA pellets could decrease the inflammatory score in the histopathological evaluation, and also it was statistically significant compared to the negative control group (p-value<0.05). No significant difference between 5-ASA pellets coated with ES100 and the positive control was observed (p-value>0.05). This data indicated that the drug release from ES100 coated pellets into colon was almost similar to that from 5-ASA pellets.

4. Conclusion

Design of experiments was successfully used to investigate the effect of process variables on the characterization of pellets. Results showed that crushing strength and mean dissolution time of pellets were strongly dependent on sodium alginate concentration and polymer ratio. Sodium alginate concentration was an essential factor in designing formulations. *In vitro* data showed that uncoated formulations failed to control the 5-ASA release and burst release was observed. Meanwhile, the incorporation of Eudragit® S100 as pH-dependent coating layer improved the controlled release of pellets. Based on the release data of pellets, F11 (20% sodium alginate and 30% Eudragit® RS coated with 10% Eudragit® S100) appears to be the best performing formulation. *In vivo* experiments revealed the therapeutic efficacy of

Eudragit® S100 coated pellets of 5-ASA in alleviating the conditions of induced colitis model as reflected by weight gain, as well as histological improvement. All these results confirmed the ability of this formulation for targeted drug delivery of 5-ASA to the colon.

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Declarations of interest

None.

References

[1] B. Tian, S. Liu, W. Lu, L. Jin, Q. Li, Y. Shi, C. Li, Z. Wang, Y. Du, Construction of pH-responsive and up-conversion luminescent NaYF 4:Yb3+/Er3+ @SiO2 @PMAA nanocomposite for colon targeted drug delivery, Sci. Rep. 6 (2016) 1–11. doi:10.1038/srep21335.

[2] S. Zakhireh, M. Mahkam, M. Yadollahi, S. Jafarirad, Investigation of pH-sensitive galactopyranoside glycol hydrogels as effective vehicles for oral drug delivery, J. Polym. Res. 21 (2014). doi:10.1007/s10965-014-0398-3.

[3] A.-M. Oprea, M.-T. Nistor, L. Profire, M.I. Popa, C.E. Lupusoru, C. Vasile, Evaluation of the Controlled Release Ability of Theophylline from Xanthan/Chondroitin Sulfate Hydrogels, J. Biomater. Nanobiotechnol. 4 (2013) 123–131. doi:10.4236/jbnb.2013.42017.

[4] A. Akhgari, M. Abbaspour, M. Moradkhanizadeh, Combination of Pectin and Eudargit RS and Eudragit RL in the Matrix of Pellets Prepared by Extrusion-Spheronization for Possible Colonic Delivery of 5-Amino Salicylic Acid., Jundishapur J. Nat. Pharm. Prod. 8 (2013) 86–92.

[5] F. Zhang, Melt-Extruded Eudragit® FS-Based Granules for Colonic Drug Delivery, AAPS PharmSciTech. 17 (2016) 56–67. doi:10.1208/s12249-015-0357-2.

[6] A. Korbely, A. Kelemen, P. Kása, K. Pintye-Hódi, Effects of Processing on the Release Profiles of Matrix Systems Containing 5-Aminosalicylic Acid, AAPS PharmSciTech. 13 (2012) 1341–1347. doi:10.1208/s12249-012-9861-9.

[7] S. Krenzlin, F. Siepmann, D. Wils, L. Guerin-Deremaux, M.P. Flament, J. Siepmann, Non-coated multiparticulate matrix systems for colon targeting, Drug Dev. Ind. Pharm. 37 (2011) 1150–1159. doi:10.3109/03639045.2011.562214.

[8] D. Hu, L. Liu, W. Chen, S. Li, Y. Zhao, A novel preparation method for 5-aminosalicylic acid loaded eudragit S100 nanoparticles, Int. J. Mol. Sci. 13 (2012) 6454–6468. doi:10.3390/ijms13056454.

[9] K. Krogars, J. Heinämäki, J. Vesalahti, M. Marvola, O. Antikainen, J. Yliruusi, J. Heinamaki, J. Vesalahti, M. Marvola, O. Antikainen, J. Yliruusi, Extrusion-spheronization of pH-sensitive polymeric matrix pellets for possible colonic drug delivery, Int. J. Pharm. 199 (2000) 187–194. doi:10.1016/S0378-5173(00)00382-3.

[10] M.W. Rudolph, S. Klein, T.E. Beckert, H.U. Petereit, J.B. Dressman, A new 5-aminosalicylic acid multi-unit dosage form for the therapy of ulcerative colitis, Eur. J. Pharm. Biopharm. 51 (2001) 183–190. doi:10.1016/S0939-6411(01)00134-5.

[11] D.S. Seeli, S. Dhivya, N. Selvamurugan, M. Prabaharan, Guar gum succinate-sodium alginate beads as a pH-sensitive carrier for colon-specific drug delivery, Int. J. Biol. Macromol. 91 (2016) 45–50. doi:10.1016/j.ijbiomac.2016.05.057.

[12] L. Li, J. Li, S. Si, L. Wang, C. Shi, Y. Sun, Z. Liang, S. Mao, ScienceDirect Effect of formulation variables on in vitro release of a water-soluble drug from chitosan e sodium alginate matrix tablets, Asian J. Pharm. Sci. 10 (2015) 314–321. doi:10.1016/j.ajps.2014.09.002.

[13] A. Mirshafiey, A. Khodadadi, B.H. Rehm, M.R. Khorramizadeh, M.B. Eslami, A. Razavi, F. Saadat, Sodium alginate as a novel therapeutic option in experimental colitis, Scand. J. Immunol. 61 (2005) 316–321. doi:10.1111/j.1365-3083.2005.01571.x.

[14] A. Razavi, A. Khodadadi, M.B. Eslami, S. Eshraghi, A. Mirshafiey, Therapeutic effect of sodium alginate in experimental chronic ulcerative colitis, Iran. J. Allergy, Asthma Immunol. 7 (2008) 13–18. doi:07.01/ijaai.1318.

[15] M. George, T.E. Abraham, pH sensitive alginate–guar gum hydrogel for the controlled delivery of protein drugs, Int. J. Pharm. 335 (2007) 123–129. doi:10.1016/J.IJPHARM.2006.11.009.

[16] H.J. Hong, J. Kim, Y.J. Suh, D. Kim, K.M. Roh, I. Kang, pH-sensitive mesalazine carrier for colon-targeted drug delivery: A two-fold composition of mesalazine with a clay and alginate, Macromol. Res. 25 (2017) 1145–1152. doi:10.1007/s13233-017-5150-5.

[17] M.R. Abbaspour, F. Sadeghi, H.A. Garekani, Preparation and characterization of ibuprofen pellets based on Eudragit RS PO and RL PO or their combination, Int. J. Pharm. 303 (2005) 88–94. doi:10.1016/j.ijpharm.2005.07.016.

[18] J. Varshosaz, J. Emami, N. Tavakoli, M. Minaiyan, N. Rahmani, F. Dorkoosh, Development and Evaluation of a Novel Pellet-Based Tablet System for Potential Colon Delivery of Budesonide, J. Drug Deliv. 2012 (2012) 1–7. doi:10.1155/2012/905191.

[19] S. Muley, T. Nandgude, S. Poddar, Extrusion–spheronization a promising pelletization technique: In-depth review, Asian J. Pharm. Sci. 11 (2016) 684–699. doi:10.1016/j.ajps.2016.08.001.

[20] J. Grund, M. Koerber, M. Walther, R. Bodmeier, The effect of polymer properties on direct compression and drug release from water-insoluble controlled release matrix tablets, Int. J. Pharm. 469 (2014) 94–101. doi:10.1016/j.ijpharm.2014.04.033.

[21] Y. Karrout, L. Dubuquoy, C. Piveteau, F. Siepmann, E. Moussa, D. Wils, T. Beghyn, C. Neut, M.-P.P. Flament, L. Guerin-Deremaux, L. Dubreuil, B. Deprez, P. Desreumaux, J. Siepmann, In vivo efficacy of microbiota-sensitive coatings for colon targeting: A promising tool for IBD therapy, J. Control. Release. 197 (2015) 121–130. doi:10.1016/j.jconrel.2014.11.006.

[22] T. Bautzová, M. Rabišková, A. Béduneau, Y. Pellequer, A. Lamprecht, Bioadhesive pellets increase local 5-aminosalicylic acid concentration in experimental colitis, Eur. J. Pharm. Biopharm. 81 (2012) 379–385. doi:10.1016/j.ejpb.2012.02.011.

[23] M. Xu, M. Sun, H. Qiao, Q. Ping, E.S. Elamin, Preparation and evaluation of colon adhesive pellets of 5-aminosalicylic acid, Int. J. Pharm. 468 (2014) 165–171. doi:10.1016/j.ijpharm.2014.04.040.

[24] H. Wei, L.F. Fan, B. Min, Y.Z. Chang, X. Bai, D. Qing, W. Feng, Q. Min, C. De-Ying,

549 Chitosan/Kollicoat SR 30D film-coated pellets of aminosalicylates for colonic drug delivery, J.
550 Pharm. Sci. 99 (2010) 186–195. doi:10.1002/jps.21810.

551 [25] Y. Karrout, C. Neut, D. Wils, F. Siepmann, L. Deremaux, M.P. Flament, L. Dubreuil, P.
552 Desreumaux, J. Siepmann, Peas Starch-Based Film Coatings for Site-Specific Drug Delivery
553 to the Colon, J. OfAppliedPolymer Sci. 119 (2010) 1176–1184. doi:10.1002/app.

554 [26] A. Akhgari, F. Sadeghi, H.A. Garekani, Combination of time-dependent and pH-dependent
555 polymethacrylates as a single coating formulation for colonic delivery of indomethacin pellets,
556 Int. J. Pharm. 320 (2006) 137–142. doi:10.1016/j.ijpharm.2006.05.011.

557 [27] G. Auriemma, T. Mencherini, P. Russo, M. Stigliani, R.P. Aquino, P. Del Gaudio, Prilling for
558 the development of multi-particulate colon drug delivery systems: Pectin vs. pectin-alginate
559 beads, Carbohydr. Polym. 92 (2013) 367–373. doi:10.1016/j.carbpol.2012.09.056.

560 [28] S. Sarkar, C.V. Liew, Moistening Liquid-Dependent De-aggregation of Microcrystalline
561 Cellulose and Its Impact on Pellet Formation by Extrusion–Spheronization, AAPS
562 PharmSciTech. 15 (2014) 753–761. doi:10.1208/s12249-014-0098-7.

563 [29] F.O. Costa, J.J.S. Sousa, A.A.C.C. Pais, S.J. Formosinho, Comparison of dissolution profiles
564 of Ibuprofen pellets., J. Control. Release. 89 (2003) 199–212. doi:10.1016/S0168-
565 3659(03)00033-6.

566 [30] A.D. Millar, D.S. Rampton, C.L. Chander, A.W.D. Claxson, S. Blades, A. Coumbe, J. Panetta,
567 C.J. Morris, D.R. Blake, Evaluating the antioxidant potential of new treatments for
568 inflammatory bowel disease using a rat model of colitis, Gut. 39 (1996) 407–415.
569 doi:10.1136/gut.39.3.407.

570 [31] C. Mura, A. Nácher, V. Merino, M. Merino-Sanjuan, C. Carda, A. Ruiz, M. Manconi, G. Loy,
571 A.M. Fadda, O. Diez-Sales, N-Succinyl-chitosan systems for 5-aminosalicylic acid colon
572 delivery: In vivo study with TNBS-induced colitis model in rats, Int. J. Pharm. 416 (2011)
573 145–154. doi:10.1016/j.ijpharm.2011.06.025.

574 [32] C.B. Appleyard, J.L. Wallace, Reactivation of hapten-induced colitis and its prevention by
575 anti-inflammatory drugs, Am. J. Physiol. Liver Physiol. 269 (1995) G119–G125.
576 doi:10.1152/ajpgi.1995.269.1.G119.

577 [33] A.H. El-Kamel, A.A.M. Abdel-Aziz, A.J. Fatani, H.I. El-Subbagh, Oral colon targeted
578 delivery systems for treatment of inflammatory bowel diseases: Synthesis, in vitro and in vivo
579 assessment, Int. J. Pharm. 358 (2008) 248–255. doi:10.1016/j.ijpharm.2008.04.021.

580 [34] N. Charoenthai, P. Kleinebudde, S. Puttipipatkhachorn, Use of Chitosan-Alginate as
581 Alternative Pelletization Aid to Microcrystalline Cellulose in Extrusion/Spheronization, J.
582 Pharm. Sci. 96 (2007) 2469–2484. doi:10.1002/jps.20855.

583 [35] H. Duan, S. Lü, C. Gao, X. Bai, H. Qin, Y. Wei, X. Wu, M. Liu, Mucoadhesive
584 microparticulates based on polysaccharide for target dual drug delivery of 5-aminosalicylic
585 acid and curcumin to inflamed colon, Colloids Surfaces B Biointerfaces. 1 (2016) 510–519.
586 doi:10.1016/j.colsurfb.2016.05.038.

587 [36] V. Jain, R. Singh, Development and characterization of eudragit RS 100 loaded microsponges
588 and its colonic delivery using natural polysaccharides, Acta Pol. Pharm. - Drug Res. 67 (2010)
589 407–415.

590 [37] H. Daemi, M. Barikani, Synthesis and characterization of calcium alginate nanoparticles,
591 sodium homopolymannuronate salt and its calcium nanoparticles, Sci. Iran. 19 (2012) 2023–
592 2028. doi:10.1016/j.scient.2012.10.005.

593 [38] Y.S.R. Krishnaiah, S. Satyanarayana, Y. V. Rama Prasad, Studies of guar gum compression-
594 coated 5-aminosalicylic acid tablets for colon-specific drug delivery, Drug Dev. Ind. Pharm.
595 25 (1999) 651–657. doi:10.1081/DDC-100102221.

596 [39] H.A. Garekani, Z.F. Moghaddam, F. Sadeghi, Organic solution versus aqueous dispersion of
597 Eudragit RS in preparation of sustained release microparticles of theophylline using spray

drying, Colloids Surfaces B Biointerfaces. 108 (2013) 374–379. doi:10.1016/j.colsurfb.2013.03.012.

[40] K. Madhusudana Rao, K.S. V Krishna Rao, P. Sudhakar, K. Chowdoji Rao, M.C.S. Subha, Synthesis and characterization of biodegradable poly (vinyl caprolactam) grafted on to sodium alginate and its microgels for controlled release studies of an anticancer drug, J. Appl. Pharm. Sci. 3 (2013) 61–69. doi:10.7324/JAPS.2013.3609.

[41] A. Akhgari, H. Afrasiabi Garekani, F. Sadeghi, Combination of inulin and time dependent polymethacrylates as a coating system to achieve colonic delivery of indomethacin, DARU J. Pharm. Sci. 17 (2009) 199–208.

[42] C.S. Leopold, Coated dosage forms for colon-specific drug delivery, Pharm. Sci. Technol. Today. 2 (1999) 197–204. doi:10.1016/S1461-5347(99)00151-0.

[43] P.K. Pawar, C. Gautam, Design, optimization and evaluation of mesalamine matrix tablet for colon drug delivery system, J. Pharm. Investig. 46 (2016) 67–78. doi:10.1007/s40005-015-0214-z.

[44] T. Pongjanyakul, S. Puttipipatkhachorn, Modulating drug release and matrix erosion of alginate matrix capsules by microenvironmental interaction with calcium ion, Eur. J. Pharm. Biopharm. 67 (2007) 187–195. doi:10.1016/J.EJPB.2006.12.009.

[45] S. Azarmi, J. Farid, A. Nokhodchi, S.M. Bahari-Saravi, H. Valizadeh, Thermal treating as a tool for sustained release of indomethacin from Eudragit RS and RL matrices, Int. J. Pharm. 246 (2002) 171–177. doi:10.1016/S0378-5173(02)00378-2.

[46] M.R. Abbaspour, F. Sadeghi, H. Afrasiabi Garekani, Thermal treating as a tool to produce plastic pellets based on Eudragit RS PO and RL PO aimed for tableting, Eur. J. Pharm. Biopharm. 67 (2007) 260–267. doi:10.1016/J.EJPB.2007.01.018.

[47] N.M. Sanchez-ballester, I. Soulairol, B. Bataille, T. Sharkawi, Flexible heteroionic calcium-magnesium alginate beads for controlled drug release, Carbohydr. Polym. 207 (2019) 224–229. doi:10.1016/j.carbpol.2018.11.096.

[48] A. Halder, S. Maiti, B. Sa, Entrapment efficiency and release characteristics of polyethyleneimine-treated or -untreated calcium alginate beads loaded with propranolol–resin complex, Int. J. Pharm. 302 (2005) 84–94. doi:10.1016/j.ijpharm.2005.06.020.

[49] F.J. Otero-Espinar, A. Luzardo-Alvarez, J. Blanco-Méndez, Non-MCC materials as extrusion-spheronization aids in pellets production, J. Drug Deliv. Sci. Technol. 20 (2010) 303–318. doi:10.1016/S1773-2247(10)50047-9.

[50] N. Thakral, A.R. Ray, D.B. Shalom, A.H. Eriksson, D. Majumdar, The quest for targeted delivery in colon cancer: mucoadhesive valdecoxib microspheres, Int. J. Nanomedicine. 6 (2011) 1057. doi:10.2147/IJN.S19561.

[51] N.K. Thakral, A.R. Ray, D.K. Majumdar, Eudragit S-100 entrapped chitosan microspheres of valdecoxib for colon cancer, J. Mater. Sci. Mater. Med. 21 (2010) 2691–2699. doi:10.1007/s10856-010-4109-2.

[52] F.-Y. Hsu, D.-S. Yu, C.-C. Huang, Development of pH-sensitive pectinate/alginate microspheres for colon drug delivery, J. Mater. Sci. Mater. Med. 24 (2013) 317–323. doi:10.1007/s10856-012-4798-9.

[53] L. Agüero, D. Zaldivar-Silva, L. Peña, M. Dias, Alginate microparticles as oral colon drug delivery device: A review, Carbohydr. Polym. 168 (2017) 32–43. doi:10.1016/j.carbpol.2017.03.033.

[54] S. Pandey, A. Mishra, P. Raval, H. Patel, A. Gupta, D. Shah, Chitosan-pectin polyelectrolyte complex as a carrier for colon targeted drug delivery, J. Young Pharm. 5 (2013) 160–166. doi:10.1016/j.jyp.2013.11.002.

[55] S.T. P. D. Kothawade, H.H. Gangurde, R. K. Surawase, M. A. Wagh, Conventional and Novel

646 Approaches for Colon Specific Drug Delivery: A Review, E-Journal Sci. Technol. 6 (2011)
647 33–56.

648 [56] A. Abinusawa, S. Tenjarla, Release of 5-Aminosalicylic Acid (5-ASA) from Mesalamine
649 Formulations at Various pH Levels, Adv. Ther. 32 (2015) 477–484. doi:10.1007/s12325-015-
650 0206-4.

651

652 [1] B. Tian, S. Liu, W. Lu, L. Jin, Q. Li, Y. Shi, C. Li, Z. Wang, Y. Du, Construction of pH-
653 responsive and up-conversion luminescent NaYF 4:Yb3+/Er3+ @SiO2 @PMAA
654 nanocomposite for colon targeted drug delivery, Sci. Rep. 6 (2016) 1–11.
655 doi:10.1038/srep21335.

656 [2] S. Zakhireh, M. Mahkam, M. Yadollahi, S. Jafarirad, Investigation of pH-sensitive
657 galactopyranoside glycol hydrogels as effective vehicles for oral drug delivery, J. Polym. Res.
658 21 (2014). doi:10.1007/s10965-014-0398-3.

659 [3] A.-M. Oprea, M.-T. Nistor, L. Profire, M.I. Popa, C.E. Lupusoru, C. Vasile, Evaluation of the
660 Controlled Release Ability of Theophylline from Xanthan/Chondroitin Sulfate Hydrogels, J.
661 Biomater. Nanobiotechnol. 4 (2013) 123–131. doi:10.4236/jbnb.2013.42017.

662 [4] A. Akhgari, M. Abbaspour, M. Moradkhanizadeh, Combination of Pectin and Eudargit RS and
663 Eudragit RL in the Matrix of Pellets Prepared by Extrusion-Spheronization for Possible
664 Colonic Delivery of 5-Amino Salicylic Acid., Jundishapur J. Nat. Pharm. Prod. 8 (2013) 86–
665 92.

666 [5] F. Zhang, Melt-Extruded Eudragit® FS-Based Granules for Colonic Drug Delivery, AAPS
667 PharmSciTech. 17 (2016) 56–67. doi:10.1208/s12249-015-0357-2.

668 [6] A. Korbely, A. Kelemen, P. Kása, K. Pintye-Hódi, Effects of Processing on the Release
669 Profiles of Matrix Systems Containing 5-Aminosalicylic Acid, AAPS PharmSciTech. 13
670 (2012) 1341–1347. doi:10.1208/s12249-012-9861-9.

671 [7] S. Krenzlin, F. Siepmann, D. Wils, L. Guerin-Deremaux, M.P. Flament, J. Siepmann, Non-
672 coated multiparticulate matrix systems for colon targeting, Drug Dev. Ind. Pharm. 37 (2011)
673 1150–1159. doi:10.3109/03639045.2011.562214.

674 [8] D. Hu, L. Liu, W. Chen, S. Li, Y. Zhao, A novel preparation method for 5-aminosalicylic acid
675 loaded eudragit S100 nanoparticles, Int. J. Mol. Sci. 13 (2012) 6454–6468.
676 doi:10.3390/ijms13056454.

677 [9] K. Krogars, J. Heinämäki, J. Vesalahti, M. Marvola, O. Antikainen, J. Yliruusi, J. Heinamaki,
678 J. Vesalahti, M. Marvola, O. Antikainen, J. Yliruusi, Extrusion-spheronization of pH-sensitive
679 polymeric matrix pellets for possible colonic drug delivery, Int. J. Pharm. 199 (2000) 187–194.
680 doi:10.1016/S0378-5173(00)00382-3.

681 [10] M.W. Rudolph, S. Klein, T.E. Beckert, H.U. Petereit, J.B. Dressman, A new 5-aminosalicylic
682 acid multi-unit dosage form for the therapy of ulcerative colitis, Eur. J. Pharm. Biopharm. 51
683 (2001) 183–190. doi:10.1016/S0939-6411(01)00134-5.

684 [11] D.S. Seeli, S. Dhivya, N. Selvamurugan, M. Prabaharan, Guar gum succinate-sodium alginate
685 beads as a pH-sensitive carrier for colon-specific drug delivery, Int. J. Biol. Macromol. 91
686 (2016) 45–50. doi:10.1016/j.ijbiomac.2016.05.057.

687 [12] L. Li, J. Li, S. Si, L. Wang, C. Shi, Y. Sun, Z. Liang, S. Mao, ScienceDirect Effect of
688 formulation variables on in vitro release of a water-soluble drug from chitosan e sodium
689 alginate matrix tablets, Asian J. Pharm. Sci. 10 (2015) 314–321.
690 doi:10.1016/j.ajps.2014.09.002.

691 [13] A. Mirshafiey, A. Khodadadi, B.H. Rehm, M.R. Khorramizadeh, M.B. Eslami, A. Razavi, F.
692 Saadat, Sodium alginate as a novel therapeutic option in experimental colitis, Scand. J.
693 Immunol. 61 (2005) 316–321. doi:10.1111/j.1365-3083.2005.01571.x.

[14] A. Razavi, A. Khodadadi, M.B. Eslami, S. Eshraghi, A. Mirshafiey, Therapeutic effect of sodium alginate in experimental chronic ulcerative colitis, Iran. J. Allergy, Asthma Immunol. 7 (2008) 13–18. doi:07.01/ijaai.1318.

[15] M. George, T.E. Abraham, pH sensitive alginate–guar gum hydrogel for the controlled delivery of protein drugs, Int. J. Pharm. 335 (2007) 123–129. doi:10.1016/J.IJPHARM.2006.11.009.

[16] H.J. Hong, J. Kim, Y.J. Suh, D. Kim, K.M. Roh, I. Kang, pH-sensitive mesalazine carrier for colon-targeted drug delivery: A two-fold composition of mesalazine with a clay and alginate, Macromol. Res. 25 (2017) 1145–1152. doi:10.1007/s13233-017-5150-5.

[17] M.R. Abbaspour, F. Sadeghi, H.A. Garekani, Preparation and characterization of ibuprofen pellets based on Eudragit RS PO and RL PO or their combination, Int. J. Pharm. 303 (2005) 88–94. doi:10.1016/j.ijpharm.2005.07.016.

[18] J. Varshosaz, J. Emami, N. Tavakoli, M. Minaiyan, N. Rahmani, F. Dorkoosh, Development and Evaluation of a Novel Pellet-Based Tablet System for Potential Colon Delivery of Budesonide, J. Drug Deliv. 2012 (2012) 1–7. doi:10.1155/2012/905191.

[19] S. Muley, T. Nandgude, S. Poddar, Extrusion–spheronization a promising pelletization technique: In-depth review, Asian J. Pharm. Sci. 11 (2016) 684–699. doi:10.1016/j.ajps.2016.08.001.

[20] J. Grund, M. Koerber, M. Walther, R. Bodmeier, The effect of polymer properties on direct compression and drug release from water-insoluble controlled release matrix tablets, Int. J. Pharm. 469 (2014) 94–101. doi:10.1016/j.ijpharm.2014.04.033.

[21] Y. Karrout, L. Dubuquoy, C. Piveteau, F. Siepmann, E. Moussa, D. Wils, T. Beghyn, C. Neut, M.-P.P. Flament, L. Guerin-Deremaux, L. Dubreuil, B. Deprez, P. Desreumaux, J. Siepmann, In vivo efficacy of microbiota-sensitive coatings for colon targeting: A promising tool for IBD therapy, J. Control. Release. 197 (2015) 121–130. doi:10.1016/j.jconrel.2014.11.006.

[22] T. Bautzová, M. Rabišková, A. Béduneau, Y. Pellequer, A. Lamprecht, Bioadhesive pellets increase local 5-aminosalicylic acid concentration in experimental colitis, Eur. J. Pharm. Biopharm. 81 (2012) 379–385. doi:10.1016/j.ejpb.2012.02.011.

[23] M. Xu, M. Sun, H. Qiao, Q. Ping, E.S. Elamin, Preparation and evaluation of colon adhesive pellets of 5-aminosalicylic acid, Int. J. Pharm. 468 (2014) 165–171. doi:10.1016/j.ijpharm.2014.04.040.

[24] H. Wei, L.F. Fan, B. Min, Y.Z. Chang, X. Bai, D. Qing, W. Feng, Q. Min, C. De-Ying, Chitosan/Kollicoat SR 30D film-coated pellets of aminosalicylates for colonic drug delivery, J. Pharm. Sci. 99 (2010) 186–195. doi:10.1002/jps.21810.

[25] Y. Karrout, C. Neut, D. Wils, F. Siepmann, L. Deremaux, M.P. Flament, L. Dubreuil, P. Desreumaux, J. Siepmann, Peas Starch-Based Film Coatings for Site-Specific Drug Delivery to the Colon, J. OfAppliedPolymer Sci. 119 (2010) 1176–1184. doi:10.1002/app.

[26] A. Akhgari, F. Sadeghi, H.A. Garekani, Combination of time-dependent and pH-dependent polymethacrylates as a single coating formulation for colonic delivery of indomethacin pellets, Int. J. Pharm. 320 (2006) 137–142. doi:10.1016/j.ijpharm.2006.05.011.

[27] G. Auriemma, T. Mencherini, P. Russo, M. Stigliani, R.P. Aquino, P. Del Gaudio, Prilling for the development of multi-particulate colon drug delivery systems: Pectin vs. pectin-alginate beads, Carbohydr. Polym. 92 (2013) 367–373. doi:10.1016/j.carbpol.2012.09.056.

[28] S. Sarkar, C.V. Liew, Moistening Liquid-Dependent De-aggregation of Microcrystalline Cellulose and Its Impact on Pellet Formation by Extrusion–Spheronization, AAPS PharmSciTech. 15 (2014) 753–761. doi:10.1208/s12249-014-0098-7.

[29] F.O. Costa, J.J.S. Sousa, A.A.C.C. Pais, S.J. Formosinho, Comparison of dissolution profiles of Ibuprofen pellets., J. Control. Release. 89 (2003) 199–212. doi:10.1016/S0168-3659(03)00033-6.

[30] A.D. Millar, D.S. Rampton, C.L. Chander, A.W.D. Claxson, S. Blades, A. Coumbe, J. Panetta, C.J. Morris, D.R. Blake, Evaluating the antioxidant potential of new treatments for inflammatory bowel disease using a rat model of colitis, Gut. 39 (1996) 407–415. doi:10.1136/gut.39.3.407.

[31] C. Mura, A. Nácher, V. Merino, M. Merino-Sanjuan, C. Carda, A. Ruiz, M. Manconi, G. Loy, A.M. Fadda, O. Diez-Sales, N-Succinyl-chitosan systems for 5-aminosalicylic acid colon delivery: In vivo study with TNBS-induced colitis model in rats, Int. J. Pharm. 416 (2011) 145–154. doi:10.1016/j.ijpharm.2011.06.025.

[32] C.B. Appleyard, J.L. Wallace, Reactivation of hapten-induced colitis and its prevention by anti-inflammatory drugs, Am. J. Physiol. Liver Physiol. 269 (1995) G119–G125. doi:10.1152/ajpgi.1995.269.1.G119.

[33] A.H. El-Kamel, A.A.M. Abdel-Aziz, A.J. Fatani, H.I. El-Subbagh, Oral colon targeted delivery systems for treatment of inflammatory bowel diseases: Synthesis, in vitro and in vivo assessment, Int. J. Pharm. 358 (2008) 248–255. doi:10.1016/j.ijpharm.2008.04.021.

[34] N. Charoenthai, P. Kleinebudde, S. Puttipipatkhachorn, Use of Chitosan-Alginate as Alternative Pelletization Aid to Microcrystalline Cellulose in Extrusion/Spheronization, J. Pharm. Sci. 96 (2007) 2469–2484. doi:10.1002/jps.20855.

[35] H. Duan, S. Lü, C. Gao, X. Bai, H. Qin, Y. Wei, X. Wu, M. Liu, Mucoadhesive microparticulates based on polysaccharide for target dual drug delivery of 5-aminosalicylic acid and curcumin to inflamed colon, Colloids Surfaces B Biointerfaces. 1 (2016) 510–519. doi:10.1016/j.colsurfb.2016.05.038.

[36] V. Jain, R. Singh, Development and characterization of eudragit RS 100 loaded microsponges and its colonic delivery using natural polysaccharides, Acta Pol. Pharm. - Drug Res. 67 (2010) 407–415.

[37] H. Daemi, M. Barikani, Synthesis and characterization of calcium alginate nanoparticles, sodium homopolymannuronate salt and its calcium nanoparticles, Sci. Iran. 19 (2012) 2023–2028. doi:10.1016/j.scient.2012.10.005.

[38] Y.S.R. Krishnaiah, S. Satyanarayana, Y. V. Rama Prasad, Studies of guar gum compression-coated 5-aminosalicylic acid tablets for colon-specific drug delivery, Drug Dev. Ind. Pharm. 25 (1999) 651–657. doi:10.1081/DDC-100102221.

[39] H.A. Garekani, Z.F. Moghaddam, F. Sadeghi, Organic solution versus aqueous dispersion of Eudragit RS in preparation of sustained release microparticles of theophylline using spray drying, Colloids Surfaces B Biointerfaces. 108 (2013) 374–379. doi:10.1016/j.colsurfb.2013.03.012.

[40] K. Madhusudana Rao, K.S. V Krishna Rao, P. Sudhakar, K. Chowdoji Rao, M.C.S. Subha, Synthesis and characterization of biodegradable poly (vinyl caprolactam) grafted on to sodium alginate and its microgels for controlled release studies of an anticancer drug, J. Appl. Pharm. Sci. 3 (2013) 61–69. doi:10.7324/JAPS.2013.3609.

[41] A. Akhgari, H. Afrasiabi Garekani, F. Sadeghi, Combination of inulin and time dependent polymethacrylates as a coating system to achieve colonic delivery of indomethacin, DARU J. Pharm. Sci. 17 (2009) 199–208.

[42] C.S. Leopold, Coated dosage forms for colon-specific drug delivery, Pharm. Sci. Technol. Today. 2 (1999) 197–204. doi:10.1016/S1461-5347(99)00151-0.

[43] P.K. Pawar, C. Gautam, Design, optimization and evaluation of mesalamine matrix tablet for colon drug delivery system, J. Pharm. Investig. 46 (2016) 67–78. doi:10.1007/s40005-015-0214-z.

[44] T. Pongjanyakul, S. Puttipipatkhachorn, Modulating drug release and matrix erosion of alginate matrix capsules by microenvironmental interaction with calcium ion, Eur. J. Pharm. Biopharm. 67 (2007) 187–195. doi:10.1016/J.EJPB.2006.12.009.

[45] S. Azarmi, J. Farid, A. Nokhodchi, S.M. Bahari-Saravi, H. Valizadeh, Thermal treating as a tool for sustained release of indomethacin from Eudragit RS and RL matrices, Int. J. Pharm. 246 (2002) 171–177. doi:10.1016/S0378-5173(02)00378-2.

[46] M.R. Abbaspour, F. Sadeghi, H. Afrasiabi Garekani, Thermal treating as a tool to produce plastic pellets based on Eudragit RS PO and RL PO aimed for tableting, Eur. J. Pharm. Biopharm. 67 (2007) 260–267. doi:10.1016/J.EJPB.2007.01.018.

[47] N.M. Sanchez-ballester, I. Soulairol, B. Bataille, T. Sharkawi, Flexible heteroionic calcium-magnesium alginate beads for controlled drug release, Carbohydr. Polym. 207 (2019) 224–229. doi:10.1016/j.carbpol.2018.11.096.

[48] A. Halder, S. Maiti, B. Sa, Entrapment efficiency and release characteristics of polyethyleneimine-treated or -untreated calcium alginate beads loaded with propranolol–resin complex, Int. J. Pharm. 302 (2005) 84–94. doi:10.1016/j.ijpharm.2005.06.020.

[49] F.J. Otero-Espinar, A. Luzardo-Alvarez, J. Blanco-Méndez, Non-MCC materials as extrusion-spheronization aids in pellets production, J. Drug Deliv. Sci. Technol. 20 (2010) 303–318. doi:10.1016/S1773-2247(10)50047-9.

[50] N. Thakral, A.R. Ray, D.B. Shalom, A.H. Eriksson, D. Majumdar, The quest for targeted delivery in colon cancer: mucoadhesive valdecoxib microspheres, Int. J. Nanomedicine. 6 (2011) 1057. doi:10.2147/IJN.S19561.

[51] N.K. Thakral, A.R. Ray, D.K. Majumdar, Eudragit S-100 entrapped chitosan microspheres of valdecoxib for colon cancer, J. Mater. Sci. Mater. Med. 21 (2010) 2691–2699. doi:10.1007/s10856-010-4109-2.

[52] F.-Y. Hsu, D.-S. Yu, C.-C. Huang, Development of pH-sensitive pectinate/alginate microspheres for colon drug delivery, J. Mater. Sci. Mater. Med. 24 (2013) 317–323. doi:10.1007/s10856-012-4798-9.

[53] L. Agüero, D. Zaldivar-Silva, L. Peña, M. Dias, Alginate microparticles as oral colon drug delivery device: A review, Carbohydr. Polym. 168 (2017) 32–43. doi:10.1016/j.carbpol.2017.03.033.

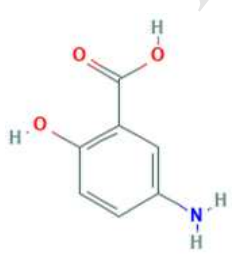
[54] S. Pandey, A. Mishra, P. Raval, H. Patel, A. Gupta, D. Shah, Chitosan-pectin polyelectrolyte complex as a carrier for colon targeted drug delivery, J. Young Pharm. 5 (2013) 160–166. doi:10.1016/j.jyp.2013.11.002.

[55] S.T. P. D. Kothawade, H.H. Gangurde, R. K. Surawase, M. A. Wagh, Conventional and Novel Approaches for Colon Specific Drug Delivery: A Review, E-Journal Sci. Technol. 6 (2011) 33–56.

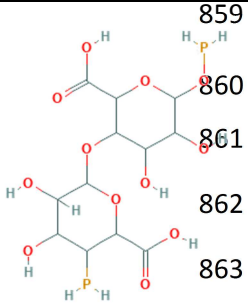
[56] A. Abinusawa, S. Tenjarla, Release of 5-Aminosalicylic Acid (5-ASA) from Mesalamine Formulations at Various pH Levels, Adv. Ther. 32 (2015) 477–484. doi:10.1007/s12325-015-0206-4.

Table 1. The solid components of pellet formulations (based on percentage)

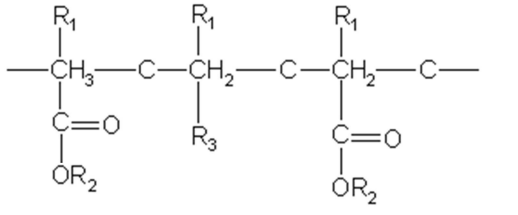
Formulation	5-ASA	Avicel	Calcium	Sodium alginate	Eudragit® RS	Eudragit® S100
F1	20	20	10	0	40	10
F2	20	20	10	0	25	25
F3	20	20	10	0	10	40
F4	20	20	10	20	24	6
F5	20	20	10	20	15	15
F6	20	20	10	20	6	24
F7	20	20	10	40	8	2
F8	20	20	10	40	5	5
F9	20	20	10	40	2	8
F10*	20	20	10	0	50	0
F11*	20	20	10	20	30	0
F12*	20	20	10	40	10	0



5-ASA



Alginate



Eudragit

866 * Eudragit® S100 in the matrix was substituted as a coating layer.

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885 **Table 2.** Experimental design

Factors employed	Level used		
	-1	0	1
X_1 = SA concentration (%)	0	20	40
X_2 = Ratio of ERS: ES100	4:1	1:1	1:4

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Table 3. The results of pellets characteristics

Formulation	The yield of pellets (%)	Image analysis	
		Aspect ratio	Sphericity
F1	89	1.22 ± 0.13	0.81 ± 0.08
F2	61	1.70 ± 0.53	0.67 ± 0.12
F3	91	1.66 ± 0.25	0.61 ± 0.09
F4	94	1.29 ± 0.16	0.78 ± 0.09
F5	94	1.23 ± 0.21	0.83 ± 0.10
F6	92	1.54 ± 0.24	0.66 ± 0.11
F7	81	1.42 ± 0.24	0.72 ± 0.12
F8	95	1.70 ± 0.30	0.49 ± 0.06
F9	94	1.56 ± 0.24	0.65 ± 0.09

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Table 4. Analysis of variance (ANOVA) of dependent variables

Source of variation	Sum of squares	df	Mean square	F value	p-value
Y_3					
Model	105.41	5	21.08	24.10	0.0126
Residuals	2.62	3	0.8747		
Total	108.03	8			
R^2 =	0.9757				
Y_4					
Model	180.36	5	36.07	11.66	0.0351

	Residuals	9.28	3	3.09		
	Total	189.64	8			
	R^2 =	0.9511				
Y_5						
	Model	1667.92	3	555.97	7.37	0.0278
	Residuals	377.40	5	75.48		
	Total	2045.32	8			
	R^2 =	0.9374				

Table 5. Predicted and experimental value of responses for selected formulations

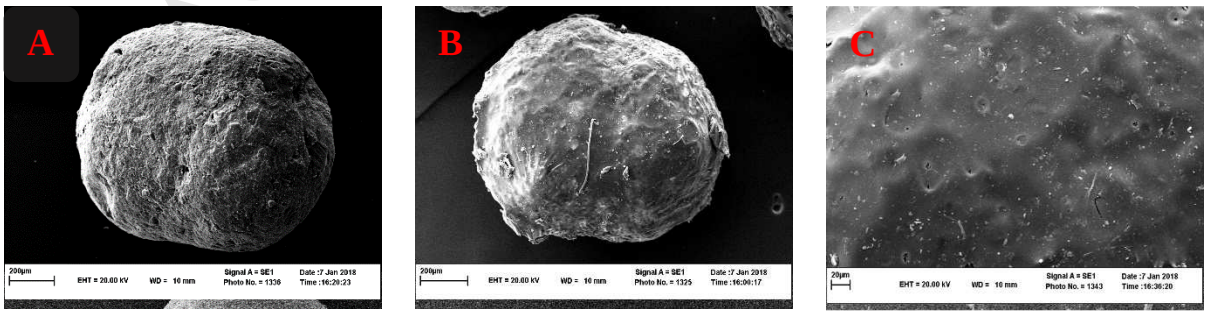
	F2			F5			F8		
	Observed	Predicted	Residual	Observed	Predicted	Residual	Observed	Predicted	Residual
Y3	4.86	4.17	0.69	4.31	5.19	-0.88	9.22	6.40	2.82
Y4	10.49	18.88	-8.39	18.24	18.53	-0.29	14.47	17.89	3.42
Y5	25.66	15.29	10.37	9.98	10.26	-0.28	6.29	5.23	1.06

Table 6. Criteria for macroscopic and histological scoring of damage

Scores	Macroscopic criteria	Histological criteria*
0	No or mild inflammation	Gablet cell depletion
1	Acute focal inflammation	Crypt abscess formation
2	Acute spread inflammation	Muscle thickening
3	Ulceration with inflammation	Cellular infiltration
4	Gangrene and necrosis	Loss of mucosal architecture

* Each of these criteria may be present or absent in these scores

Figure 1. *In vivo* rat study design: Colitis was induced on day 0 (except for normal control groups). Oral administration of the different formulations began on day 5. All animals received standard chow (standard laboratory feeding pellets).



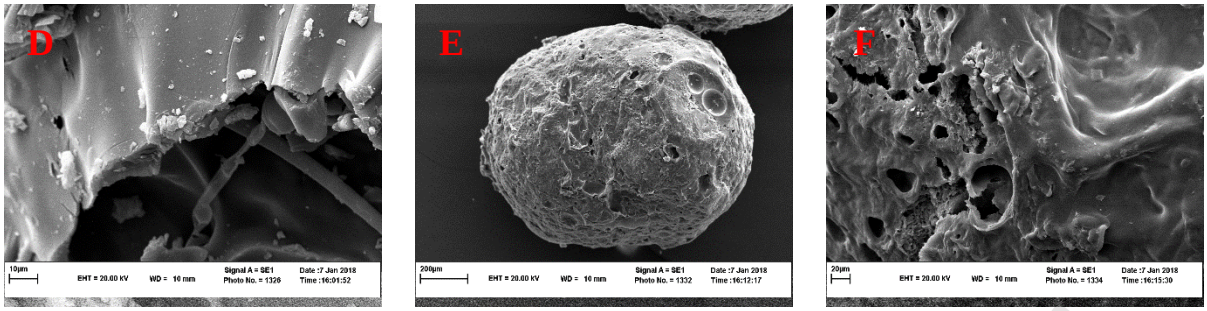


Figure 2. SEM of uncoated (A) and coated pellet at 250× (B) and 2500× (C), the thickness of the coating (D) at 3000×, coated pellet after continuous dissolution test at 250× (E) and 2500× (F) magnifications.

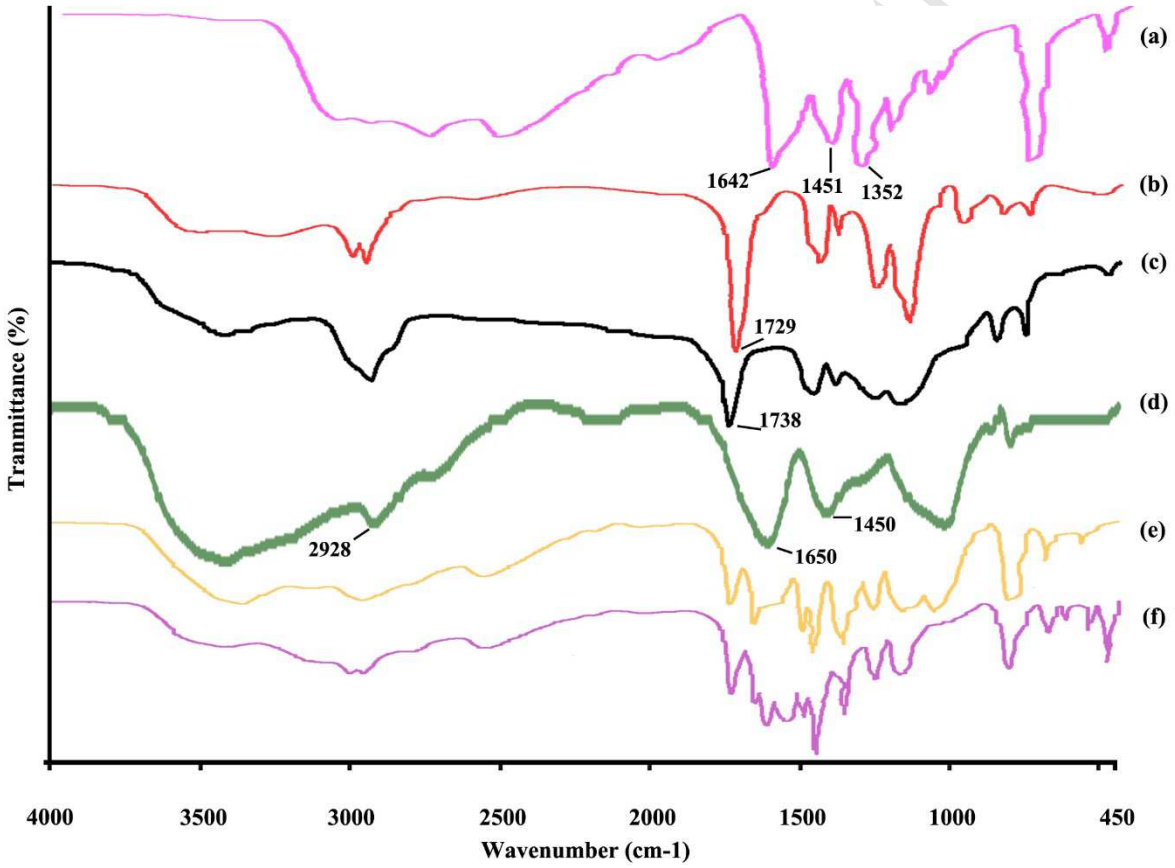
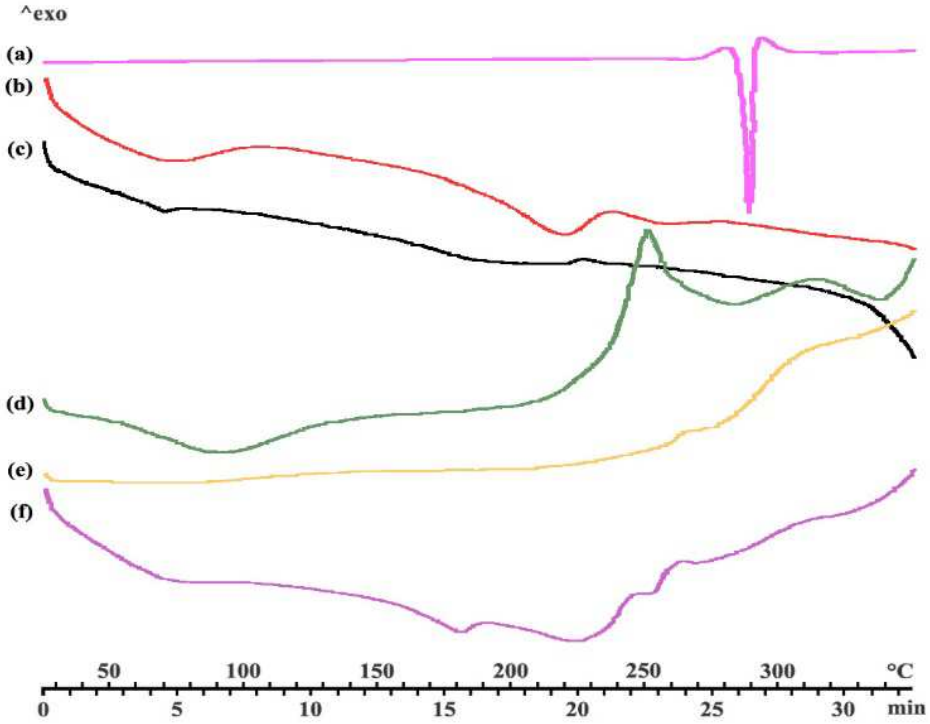


Figure 3. FTIR Spectra of drug, polymers, and physical mixture of drug and polymers. (a) 5-ASA, (b) ES100, (c) ERS, (d) alginate, (e) pellet and (f) physical mixture of ES100, ERS and alginate polymers.



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984 **Figure 4.** DSC curves of (a) 5-ASA, (b) ES100, (c) ERS, (d) alginate, (e) pellet and (f) physical
985 mixture of ES100, ERS and alginate polymers.

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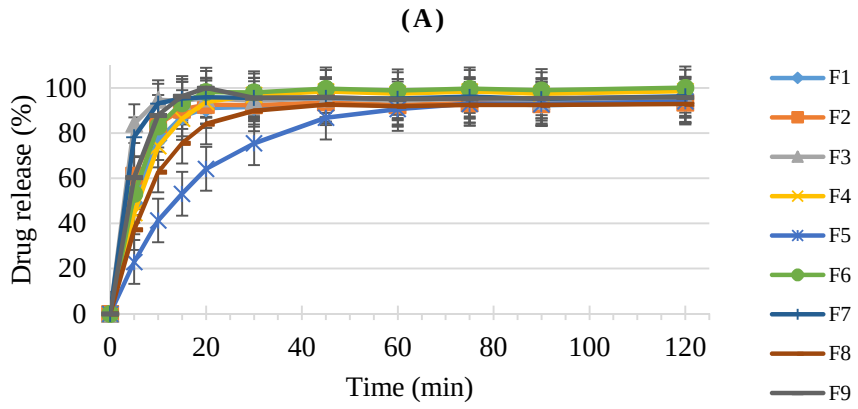


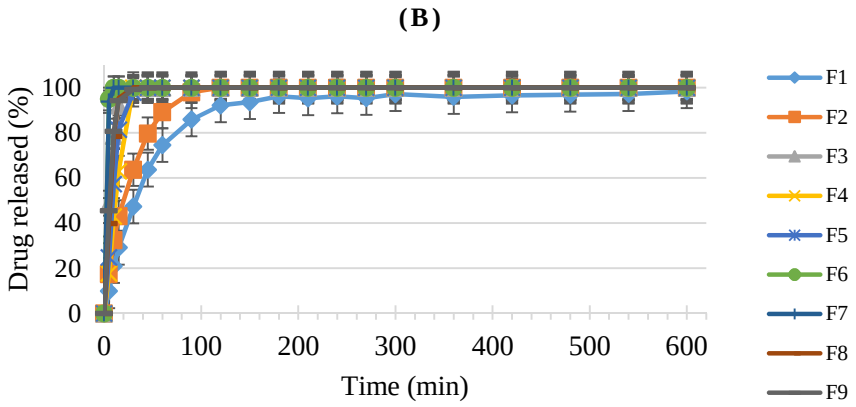
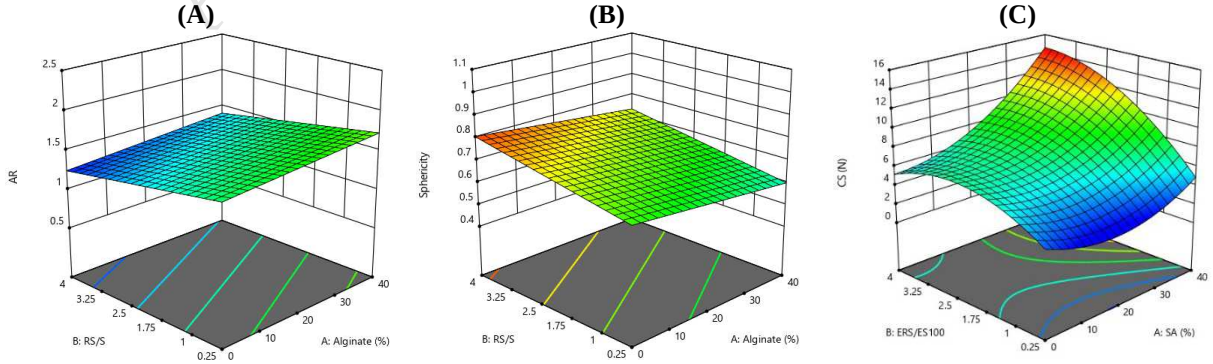


Figure 5. The dissolution profile of uncoated formulations in the media with pH 1.2 (A) and 6.8 (B).



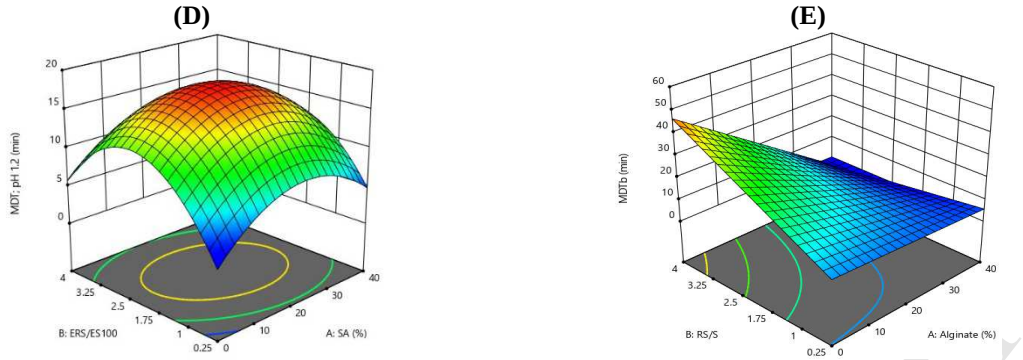
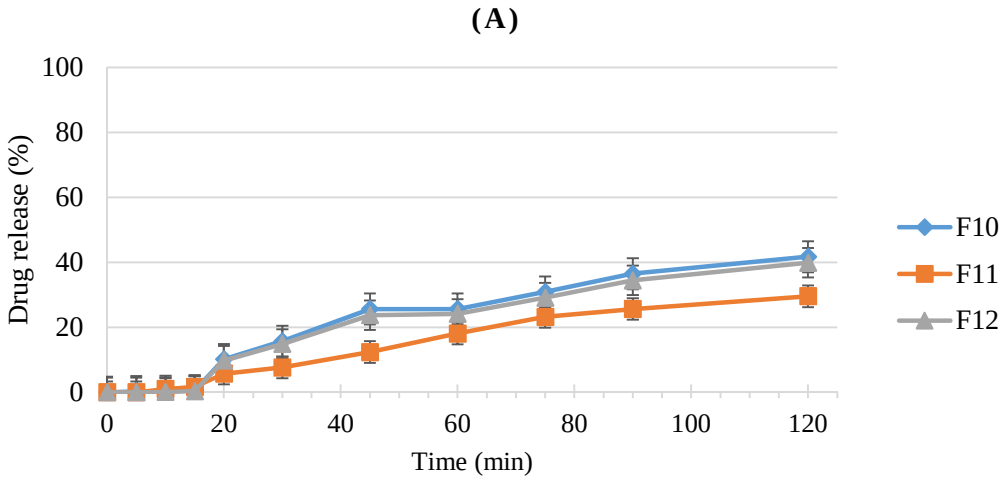
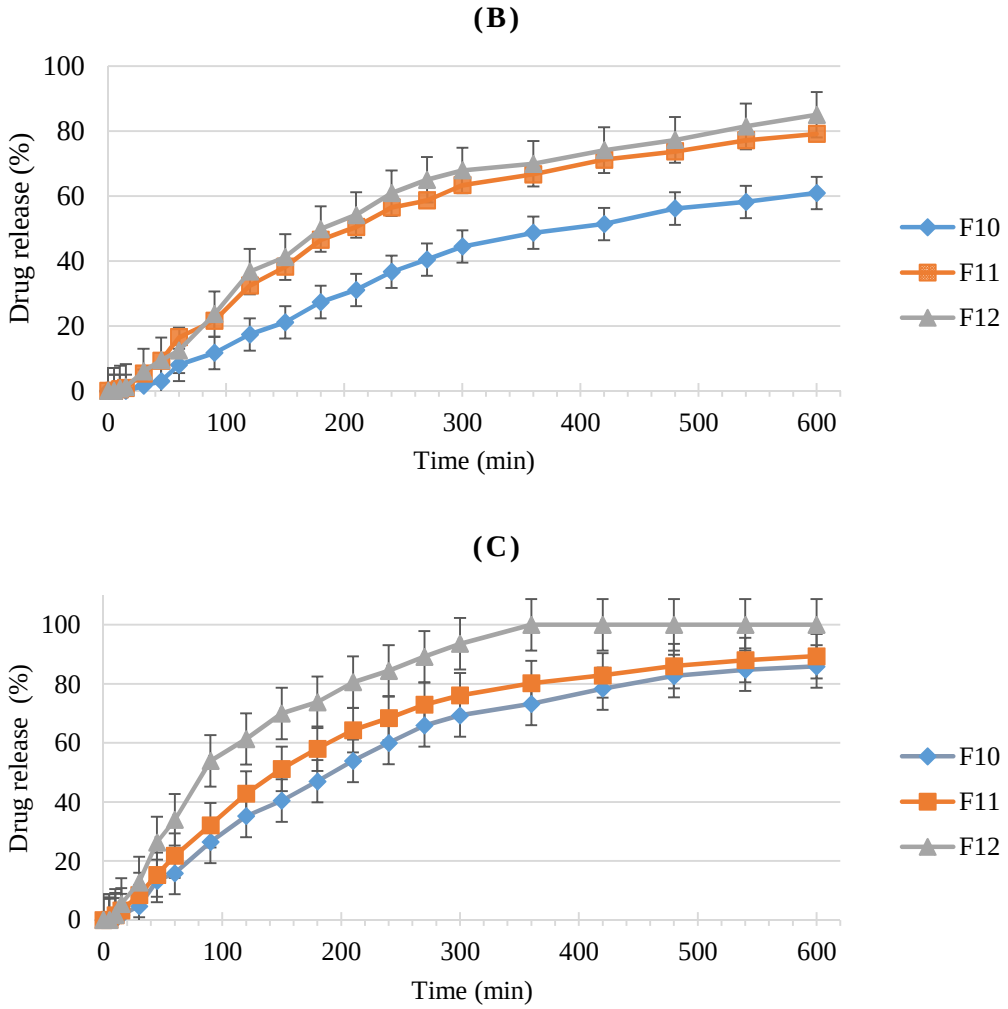


Figure 6. Response surface plot is showing the influence of sodium alginate concentration and polymer ratio on the aspect ratio (A), sphericity (B), crushing strength (C) and mean dissolution time in pH 1.2 (D) and 6.8 (E).





1012 **Figure 7.** The dissolution profile of coated formulations in the media with pH 1.2 (A), 6.8 (B) and 7.4
1013 (C)

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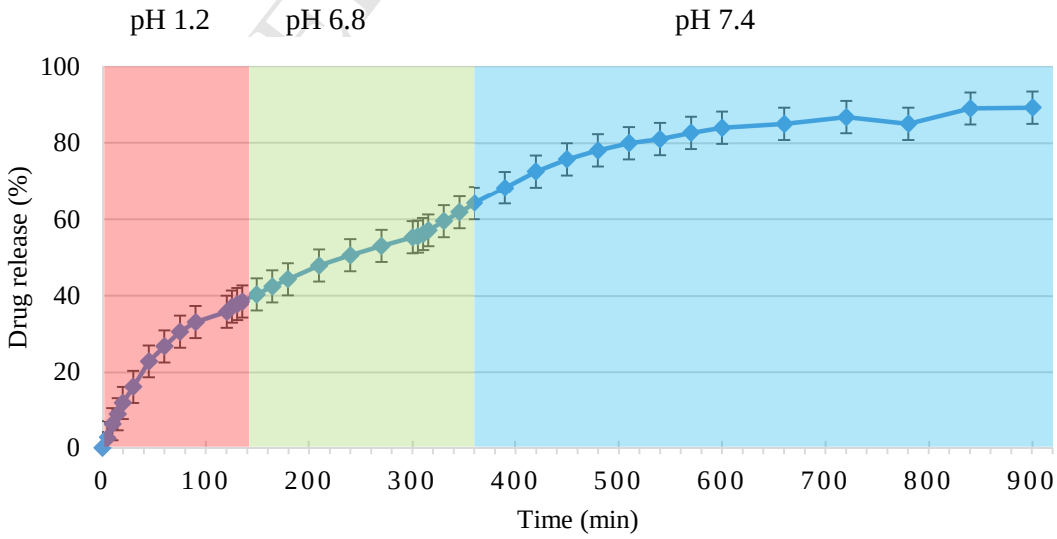

1015 **Figure 8.** The continuous dissolution profile of coated F11.

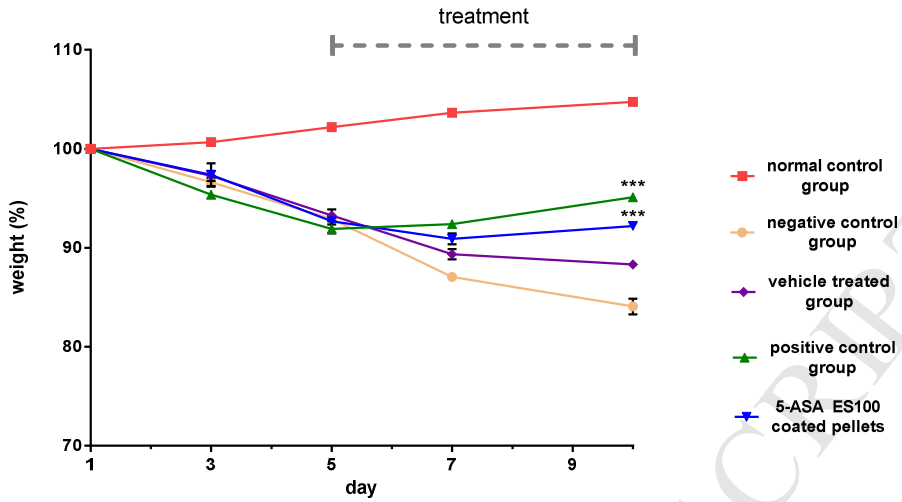

Figure 9. Determination of weight loss (mean± SEM) during the whole experimental period for n=5 animals in each group. ***p-value<0.001 compared to control negative group.

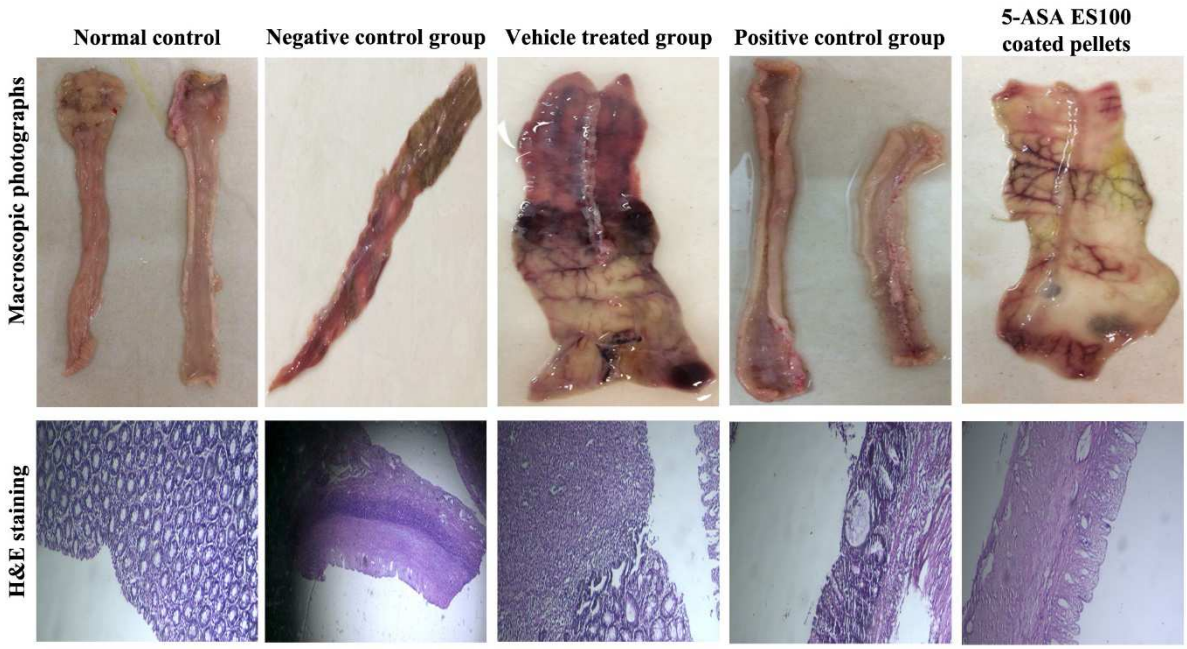

Figure 10. Representative macroscopic and histological appearance of rat colonic mucosa. The colon samples of normal (non-colitis) and negative control groups, rats receiving vehicle (1% Na CMC), 5-ASA (positive control group) and 5-ASA ES100 coated pellets were collected for histological analysis in the final day of treatment. Macroscopic photographs (upper panel) and microscopic appearances after H&E staining (below panel) of the colon samples were shown. Magnification was equivalent for all macroscopic images and 20× for H&E staining images.

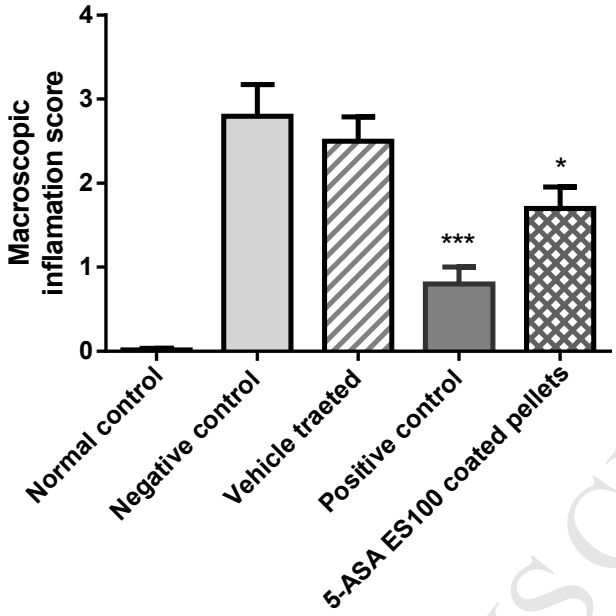

Figure 11. Histopathological inflammation scores determination (mean±SEM) for n=5 animals in each group on the final day (day10). ****p*-value<0.001,**p*-value<0.05 compared to negative control group.

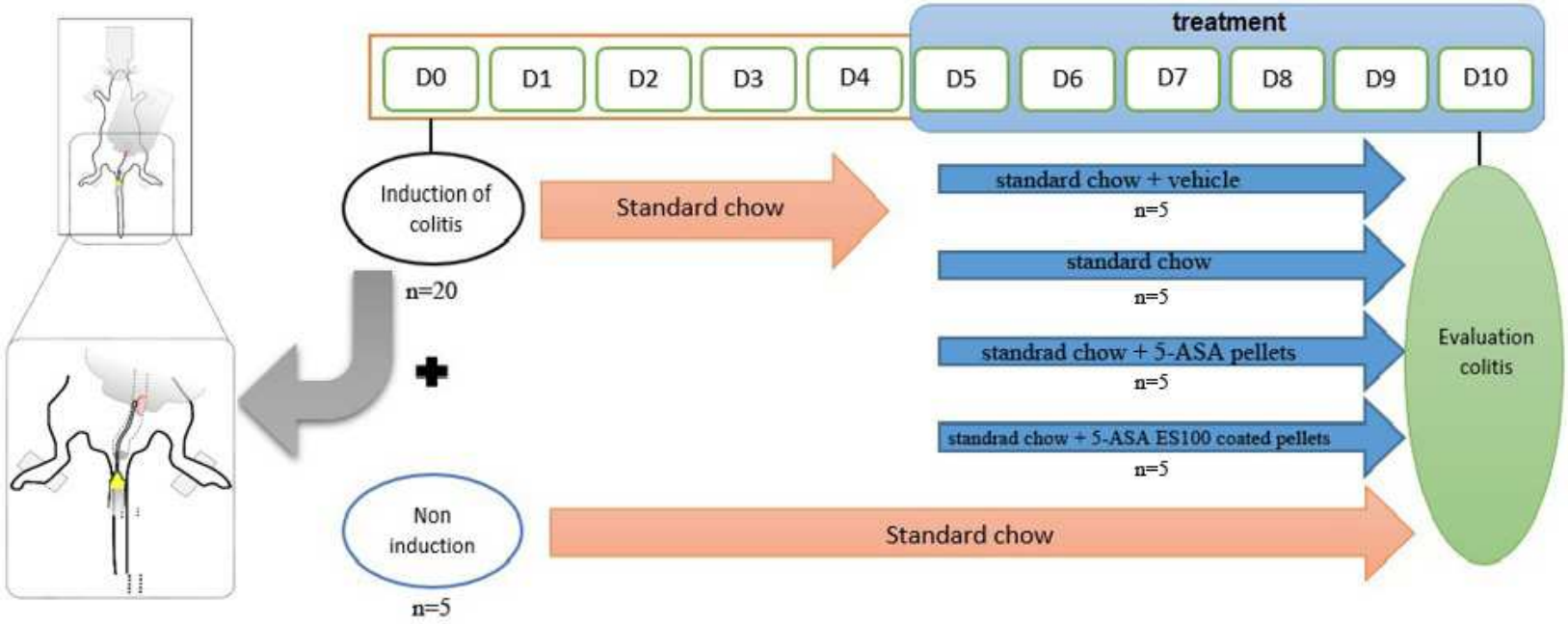

Figure 1. *In vivo* rat study design: Colitis was induced on day 0 (except for normal control groups). Oral administration of the different formulations began on day 5. All animals received standard chow (standard laboratory feeding pellets).